FORMULATION AND EVALUATION OF P^H TRIGGERED IN SITU GELLING SYSTEM OF LEVOFLOXACIN

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI

in partial fulfillment of the requirement for the award of degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

March – 2010

DEPARTMENT OF PHARMACEUTICS

COLLEGE OF PHARMACY

MADURAI MEDICAL COLLEGE

MADURAI - 625020.

FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLES OF RAMIPRIL

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Mrs.R. Tharabai, M.Pharm,
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CERTIFICATE

This is to certify that the Dissertation entitled **"FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLES OF RAMIPRIL"** submitted by **Mr. P. Ekambaram** in partial fulfillment of the requirement for the degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by him, under the guidance and supervision of **Mr.A.Abdul Hasan Sathali**, Professor and Head, in the Department of Pharmaceutics, during the academic year 2009 – 2010, Madurai Medical College, Madurai-20.

I wish him success in all his endeavors.

Place: Madurai

Date: **(Mrs.R.Tharabai)**

DESIGN AND CHARACTERIZATION OF TRANSDERMAL DELIVERY OF REPAGLINIDE

Dissertation submitted to

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FORMULATION AND EVALUATION OF DICLOFENAC POTASSIUM ETHOSOMES.

Dissertation submitted to

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CHAPTER-I

INTRODUCTION

During the past decade, advanced drug delivery research and development has surged. The emergence of nanotechnology and the growing capabilities of proteomics, genomics and combinatorial chemistry have provided scientists with new technologies. Commonly accepted criteria of advanced drug delivery system include [1]:

a) maximal drug bioavailability

b) tissue targeting

c) controlled release kinetics

d) minimal immune response

e) ease of administration for patient compliance

f) ability to delivery different drug such as lipophiles, amphiphiles and biomolecules.

A drug's therapeutic efficacy depends on four fundamental pathways of drug transport and modification within the body:

- absorption into the plasma form the administration site.
- distribution between plasma and tissues.
- metabolism within the tissues.
- elimination from the body.

Absorption rate depends on many factors such as hydrophobicity, chemical environment, particles size, crystallinity, blood flow, absorptive surface area, and resident time at absorptive surface [2].

Drug distribution largely depends on blood flow, capillary permeability such as in BBB, ligand binding and hydrophobicity.

Drug metabolism and elimination primarily depend on afore mentioned properties. The drug delivery system can greatly impact each pathway, and, therefore, the delivery system is a critical design component in pharmaceutical sciences.

Lipophiles (or) poorly water soluble drug, perform pivotal rotes in numerous biological processes. Many leadingsmall molecules drug are lipophilic. Anticancer drug including piposulfan, etoposide, camptothecin and paclitaxel are lipophilic. Antifungal drugs such as amphotericin-B, fluconazolo, itraconazoie are lipophilic. Key antioxidants such as vitamin A, vitamin E, retinol, lycophene and β-carotene also are lipophilic. These lipophiles must be formulated and delivered in a safe, efficacious, and cost effective manner. Lipophile delivery is extremely challenging and has long been a source of frustration in the pharmaceutical sciences. So, it has become more and more evident that the development of new drug alone is not sufficient to ensure progress in drug therapy.

Main reasons for failure in therapy include [3]:

- Insufficient drug concentration due to poor absorption, rapid metabolism, and elimination. Drug distribution to other tissues combined with high drug toxicity.
- Poor drug solubility which excludes iv injection of aqueous drug solution.
- High fluctuation of plasma levels due to unpredictable bioavailability after per oral administration, including the influence of food on plasma levels.

A promising strategy to overcome these problems involves the development of suitable drug colloidal carrier system.

NEED OF COLLOIDAL CARRIER SYSTEMS [57]

Colloidal carriers have attracted the main interest because they are promising systems to fulfill the requirements of a poorly aqueous soluble drug. But, in the first

place, nanosized carriers are treated as hopeful means to increase the solubility and therefore the bioavailability of poorly water soluble –activity ingredients belonging to the classes II and IV in the biopharmaceutical classification system [BCS] [3, 4].

The common characteristic of all colloidal carriers is the sub-micron particle size. Corresponding to the broad diversity of colloidal carriers, the possible administration routes vary

- Dermal
- Peroral
- Parenteral
- Ocular
- Pulmonary application

As upper limit for IV administration to avoid embolism in blood vessels no particles above 5µm and only few particles between one to five micrometers are accepted. Solid particular systems are limited to either the subcutaneous (or) intramuscular routes of administration; intravenous administration may result in vaso occlusion.

Focusing on the biofate of lipid – containing drug carriers after per oral administration, short chain and medium chain liquid lipids are known to be easily hydrolyzed and to be readily absorbed in the gastrointestinal tract [GIT]. Crystalline lipids are poorly attached by lipases and very long chains (From c18 up) in solid state are poorly absorbed. Often penetration and uptake of entire colloidal particles in cell tissues is not probable to explain the in vivo effects of colloidal surface area of nanoparticles compared to micro particles, Eg: improved drug solubility and therefore better bioavailability is given [5].

Nanosizing of the bulk material may also lead to dramatic changes of the physical properties of the substance,(ie) the depression of the melting point

which results in the existence of super cooled melts. Therefore colloids are not trivial system. furthermore, different colloidal structures might co-exit.

Of course, nanometric systems have to fulfill the request's for save drug delivery systems mentioned above. Most of all, precautions against aggregation, coalescence. Additionally incorporation of sensitive drug molecule in some carrier matrices is claimed as protection against enzymatic degradation, hydrolysis (or0 light. Despite of their small size, colloidal carriers have to controlled drug release.

COLLOIDAL DRUG CARRIER SYSTEMS

A. NANOCAPSULES [6, 7]

Oil containing nanocapsules differ from nanoemulsions in providing a barrier made from polymer between the core and the surrounding environment, but as well nanoparticles with aqueous cores in an aqueous outer phase are published. Often, for the preparation of nanocapsules the way of solvent displacement and interfacial polymerization are applied.

According to the liphophilicity of the capsule content, hydrophilicity and lipophilic drugs can be dissolved additionally, the polymeric particle surface may serve as compartment lipophilic drugs have already shown to be released in t\a controlled manner. Encapsulation may decrease the toxicity of drugs after peroral (or) parenteral application in as much as the exhibition to cells is diminished. Encapsulation saves sensitive drugs from rapid degradation with the aim to reduce the interactions with reticuloendothetial system (RES) and to alter body distribution; the surface of nanocapsules was modified by certain materials such as surfactants.

B. NANOSUSPENSIONS [8, 9]

Nanosuspensions are saturated solutions. They represent the simplest colloidal carriers with respect to composition. The drug payload amounts to nearly 100%. In an aqueous environment, the drug is pearl milled, precipitated (or) high pressure homogenized to a particle distribution mostly below one micrometer. Despite of the use of tensides , particle growth up to micrometric drug crystals may occur when the drug molecules of small particles dissolve in the outer environment and precipitate later on the surface of larger particles.

Due to tremendous interface area between drug and environment, solubilization velocity of the drug is increased according to the rays Whitney equation. Attention has to be paid on drugs with small safety margins where burst release has to be avoided. But controlled reease and reproducible blood levels are not easily achievable because as a release controlling barrier only the tenside layer may serve in these nanosuspensions. Suspensions of crystals in the μm range are already established in the martiet (eg: predigaleu ^{TM}). The only two registered nanosuspensions are repamune ^{TM} and emend ^{TM} for immediate delivery.

C. LIPOSOMES [10, 11]

Liposomes consists of one (or) more lipid bilayers of amphiphilic lipids(ie; Phospholipids, cholesterol, glycolipids). The lipophilic moiety of the bilayer is turned towards each other and creates an inner hydrophobic environment in the membrane. Lipophilic (or) amphiphilic drugs can be associated with non polar parts of lipid bilayers if they fit in size and geometry.the hydrophilic molecular head groups face the outer water phase and the inner aqueous core of the vesicles. Water soluble compounds can be included within the aqueous compartments.

Liposomes are classified as large multilamellar liposomes (MLV), large unilameller vesicles(LUV), small unilameller vesicles (SUV), oligolameller vesicles (OLV) and multivesicular (MVU), depending on the their size, the number of bilayers and the existence of inner vesicles in a vesicle. The size of liposomes varies from 20nm to few micrometers with lipid membranes approximately 5nm.

Some of the marketed products are

- Ambisome ^{TM} -parenteral
- Daunoxome ^{TM}-parenteral
- Prvaryl ^{TM}- Lipogel- Topical administration.

D. MIXED MICELLES [10, 12]

As long – chain phospholipids are known to form bilayers when dispersed in water, the preferred phase of short-chain analogues is the micellar phase. In general ampholytic molecules, which are able to decrease the surface tension of a solvent, arrange in micelles, as Tween ^{TM} (or) sodium dodecyl sulfate above a certain critical concentration. A micellar solution is a thermodynamically stable system formed spontaneously in water and also in organic solvents. The latter is of less interest in pharmaceutical technology solute concentration, the critical micellar concentration (CMC) and at solution temperatures above the critical micellar temperature (CMT). The small colloidal aggregates (micelles) are in rapid thermodynamic equilibrium with a measurable concentration of monomers micellar solutions. Exhibit solubilization phenomena. The micelle solubilizes last molecules (ie. Drugs) in any zone of the micelle volume, but the penetration into the micelle depends over all on the inner space of the micelle, on the hydrophobicity of the drug and on the charge of the incorporated molecule. The interaction between micelles and lipophilic drugs leads to the formation

of mixed micelles (MM), often called as swollen micelles, too the addition of salt, alcohol, etc can vary the degree of penetration into the micelle (Co- solubilization). In mixed micelles, the mobility of the micellar phase was found to be decreased due to incorporate molecules. Considerably swollen micelles are larger than the analogous "free micelles" because solubilization may result mostly from the increase in micellar site.

E. COLLOIDAL LIQUID CRYSTALLINE STRUCTURES [13]

Liquid crystalline phases share features from both liquids and crystalline substances. Due to their intermediate state they are named as "meso phase", too on one hand, referring to crystal related phenomena they can be characterized by diffraction scanning calorimetry (DSC), X-ray diffraction and polarization microscopy. On the other hand, liquid crystals match partially self-organized melts in providing remarkable viscosity and diffusion characteristics.

DISADVANTAGE [13]

The tenside concentrations are high and that colloidal dispersion of liquid crystals occur only in a thin range of parameters. Mesophases are thermodynamically stable and self-assembling, but they form reversible the former based micellar (or) molecular dispersed state by adding water.

F. MICROEMULSIONS [14]

Microemulsions are optically isotropic, transparent (or) translucent, low-viscous, single phasic and thermodynamically stable liquid solution. Microemulsions are

bicontinuous systems that are essentially composed of water and oil, separated by surfactant and co-surfactant.

Due to their large interfacial areas microemulsions typically show. Much greater solubilizing capacities for both hydrophilic and lipophilic drugs than micellar solution. Microemulsions are limited to dermal and per oral administration because of their high surfactant concentration. They exist in narrow regions of phase diagrams; therefore they are very restricted in tolevance to quantitative formulation change.

DISADVANTAGE [14]

Microemulsions are unable to dilute and therefore size determination sometimes is difficult.

G. NANOEMULSIONS [9]

Nanoemulsions are heterogenous systems comprised if two immiscible liquid is dispersed as droplets in another liquid. O/W nanoemulsions present the most important parenteral drug carrier systems where lipophilic drug are dissolved in the inner phase of the emulsion. Degradation of the droplets containing lipophilic drug occurs very fast when administered intravenously, so retarded release is not realized. With regard to the mobility of the oil a protection of sensitive drug molecules from hydrolysis is hindered. Moreover sustained release and incorporation of hydrophilic components in conventional oil in water is not realizable. Multiple emulisions (w/o/w) are proposed to resolve these problems.

DISADVANTAGES [9]

1) Rapid degradation for oil droplets takes place in GIT if given per orally.

2) Unstable systems

3) Difficult to handle

H. POLYMER NANOPARTICLES [15]

Polymers suitable for the preparation of nanoparticles include cellulose derivatives, poly (alkyl cyanoacrylates) poly (methylidene malonate), polyorthoesters, polyanhydrides and polyesters such as poly (lactic acid), poly (glycolic acid) and poly (t-caprolactone) and their copolymers. Polymers used for parenteral delivery have to be biodegradable; they mostly belong to polyesters (or) to the group of polyacrylates. For Peroral administration, non-degradable polymers such as acrylate-and cellulose-derivatives can be used for nanoparticles designed not be absorbed.

For the production of polymer nanoparticles various procedures are applied:

- Coacervation technique
- Solvent evaporation
- Solvent diffusion
- Interfacial polymerization
- Denaturation of nature proteins
- Degradation by high shear forces
- Microfludization

Depending on the polymer, drug and polymer interaction production procedure, drug release differs.

I. SOLID LIPID NANOPARTICLES AND NANOSTRUCTURED LIPID CARRIERS [16, 17]

Melt- emulsified nanoparticles based on lipids (or) waxes solid at room temperature have been developed.

ADVANTAGES [16]

- Use of physiological well tolerated lipids
- Avoidance of organic solvents
- Wide potential application spectrum.
- avoid toxicity
- Improved bioavailability
- Protection of drug molecules
- Controlled release characteristics

DISADVANTAGES [16]

- Particle growth
- Particle aggregation
- Unpredictable gelation tendency
- Unexpected dynamics of polymorphic transitions
- Burst drug release
- Inherently low incorporation capacities due to the crystalline structure of lipid.

NANOSTRUCTRED LIPID CARRIERS (NLC) [17]

Also known as oil loaded solid lipid nanoparticles. Liquid lipid solubilizes drug much to a higher extent than solid lipids.

ADVANTAGES [17]

- High incorporation capacity (due to the liquid lipid)

- Control of drug release (due to the encapsulating solid lipid)

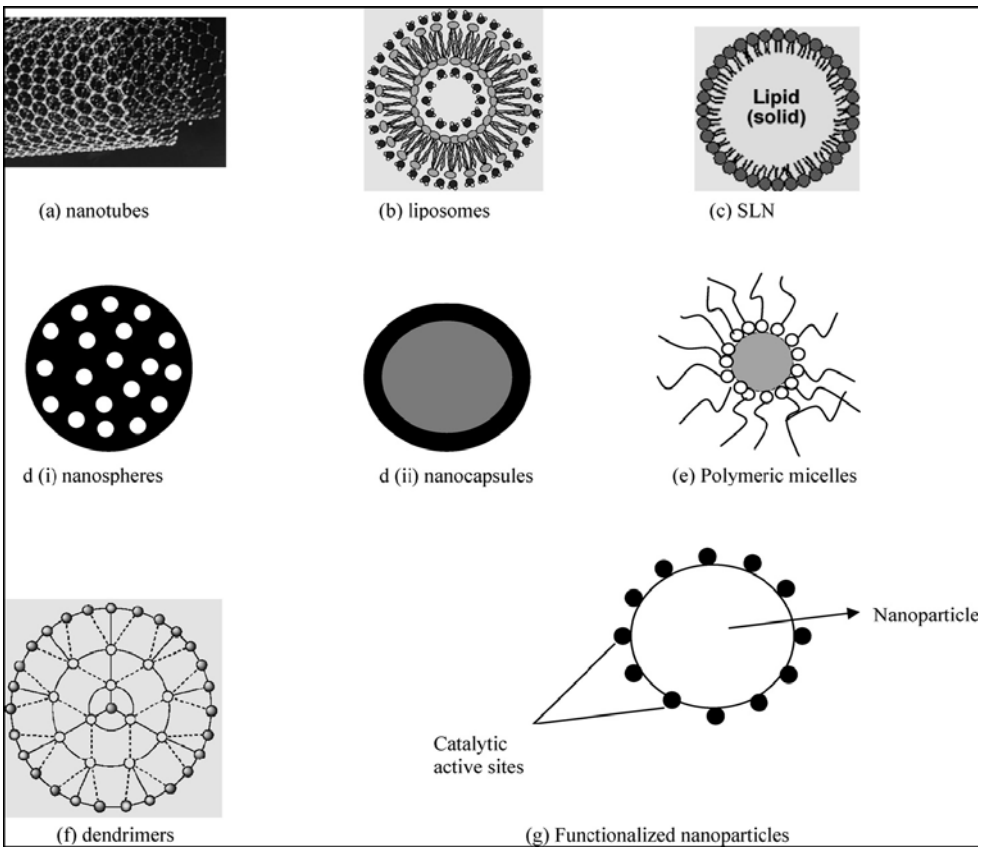


Figure - 1
Different Types of Nanocarriers

(a) Nanotubes: self-assembling lipid tubes. (b) Liposomes: concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer. (c) Solid lipid nanoparticles (SLN): submicron colloidal carriers made from solid lipids. (d) Polymeric nanoparticles. (i) Nanospheres: nanoparticles in which drug is dispersed through out the polymeric matrix. (ii) Nanocapsules: nanoparticles in which drug is encapsulated within polymeric membrane. (e) Polymeric micelles: amphiphilic block copolymers that self-associate in aqueous solution. (f) Dendrimers: macromolecular compounds that consist of a series of branches around an inner core. (g) Functionalized nanoparticles: monodisperse-sized particles of uniform shape with well-defined surface composition.

CHAPTER-II

SOLID LIPID NANOPARTICLES (SLN)

Solid lipid nanoparticles typically are spherical with average diameter between 1 to 1000nm [16, 18]. Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to tradition colloidal carriers such as –emulsions, liposomes and polymeric micro-and nanoparticles. They are a new generation of submicron-sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid.

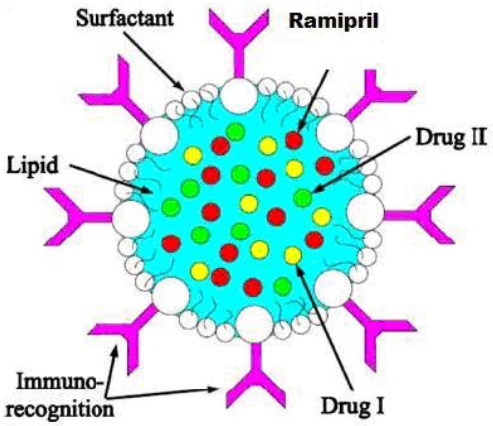


Figure – 2

Schematic representation of a Solid lipid nanoparticle [55]

SLN offer unique properties, such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improved performance of pharmaceuticals. In 1980, speiser and coworkers were the first to report making solid lipid particles for drug delivery applications. The reasons for the increasing in lipid based system are many-fold and include [16, 17, 19]

1) Lipids enhance oral bioavailability and reduce plasma profile variability.
2) Better characterization of lipoid excipients.
3) An improved ability to address the key issues of technology transfer and manufacture scale-up.

SOLID LIPID NANOPARTICLES (SLN) INGREDIENTS [20]

General ingredients include solid lipid(S), emulsifier(S) and water. The term lipid is used generally in a very broad sense and includes triglycerides (eg. Tristearin, hard fat), partial glycerides (eg. Imwitor), pegylated lipid, fatty acid (stearic acid), steroids (e.g. cholesterol) and waxes (eg. Cetyl palmitate). Surfactants investigated include biological membrane lipids such as lecithin, bile salts such as sodium taurocholate, biocompatible non ionics such as ethylene oxide / propylene oxide copolymers, sorbitan esters, fatty acid and ethoxylates and mixtures there of. All classes of emulsifiers have been used to stabilize the lipid dispersion. The most frequently used compounds include different kinds of poloxamer, polysorbates, lecithin and bile acids. It has been found that the combination of emulsifiers might prevent particle agglomeration more efficiently.

LIPIDS	**SURFACTANTS**
TRIACYLGLYCEROLS	**PHOSPHOLIPIDS**
Tricaprin	Soy lecithin
Trilaurin	Egg lecithin
Trimyristin	Phosphotidylcholine
Tripalmitin	**ETHYLENE OXIDE / PROPYLENE COPOLYMERS**
Tristearin	Poloxamer 188

	Poloxamer 182
ACYL GLYCEROLS	Poloxamer 407
Glyceryl monostearate	Poloxamer 908
Glyceryl behanate	**SORBITAN ETHYLENE OXIDE / PROPYLENE OXIDE COPOLYMERS**
Glyceryl palmitostearate	
FATTY ACIDS	Polysorbate 20
Stearic acid	Polysorbate 80
Palmitic acid	Polysorbate 60
Deconoic acid	**ALKYL ARYL POLYETHER ALCOHOL POLYMERS**
Behanic acid	Tylopaxal
WAXES	**BILE SALTS**
Cetyl palmitate	Sodium cholate
CYCLIC COMPLEXES	Sodium glycolate
Cyclodextrin	Sodium tauracholate
Para – acyl – calyx - arenes	Sodium taurodeoxy cholate
	ALCOHOLS
	Ethanol
	Butanol

Lipids and surfactants used in the solid lipid nanoparticle production

Methods of SLN preparation

1. High shear homogenization [21, 22]

High shear homogenization technique was initially used for the production of solid lipid nanodispersion.

a) Hot homogenization [21]

Hot homogenization is carried out at temperatures above the melting point of the lipid and is similar to the homogenization of an emulsion. A pre emulsion of the drug loaded lipid melt and the aqueous emulsifier phase is obtained by high shearing mixing device. The quality of the pre emulsion affects the final product. High pressure homogenization of the pre emulsion done above the lipid melting point. Usually lower particle sizes are obtained at high viscosity of the liquid phase. Increasing the homogenization leads to an increase of the particle size due to particle coalescence, this occurs because of kinetic energy of the particles.

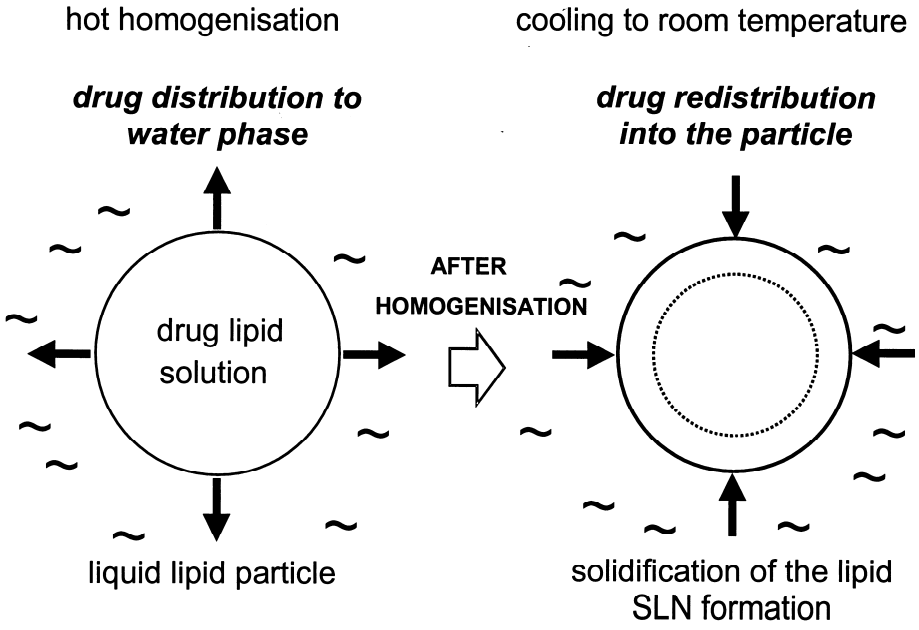


Figure - 3

Partitioning effects on drug during the production of SLN by the hot homogenization technique. Left: Partitioning of drug from the lipid phase to the

Water phase at increased temperature. Right: Re-partitioning of the drug to the lipid phase during cooling of the produced O/W nanoemulsion.

b) Cold homogenization [22]

The cold homogenization process is carried out with the solid lipid and there fore is similar to milling of a suspension at elevated pressure. Cold homogenization has been developed to overcome the followed problems of the hot homogenization technique such as: temperature mediated accelerated degradation of the drug pay load, partitioning and hence loss of drug into aqueous phase during homogenization, uncertain polymorphic transitions of the due to complexity of the crystallization step of the nanoemulsion leading to several modifications (or) super cooled melts.

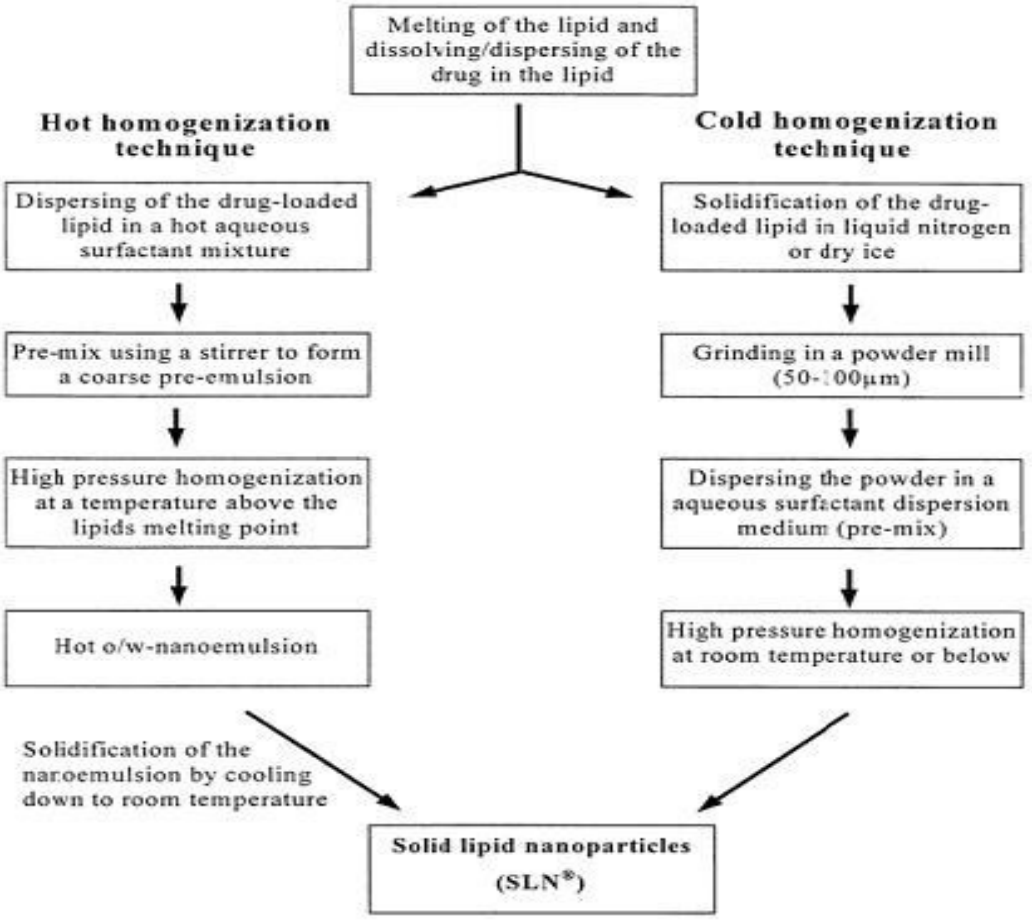


Figure - 4
Comparison of hot and cold high pressure homogenization processes

2. **Ultrasonication (or) high speed homogenization [23]**

SLN were also developed by high speed stirring (or) sonication, a most advantages are that, equipments that are used here are easily available in every lab. The problem of this method is broader particle size distribution ranging in micrometer range. This lead physical instability likes particle growth upon storage. Potential metal contamination due to ultrasonication is a big problem in this method. So for making a suitable formulation studies have been performed at high temperature.

3. SLN prepared by solvent evaporation / emulsification [24]

For the production of the nanoparticle dispersions by precipitation of o/w emulsions, the lipophilic material is dissolved in which water – immiscible organic solvents (cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The mean diameter of the obtained particles was 25nm with cholesterol acetate as model drug.

4. Microemulsion based SLN preparation [25, 26]

Gasco and co workers developed SLN preparation techniques which are based on the dilution of the microemulsions. They are made by stirring an optically transparent mixture at 65 - 70°C which is typically composed of a low melting point fatty acid (stearic acid), an emulsifier (tween 20) and co emulsifier and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring. Typical volume ratios of the microemulsion to cold water are in the range 1:25 and 1:50. The dilution process is critically determined by the composition of the microemulsion.

5. SLN preparation by super critical fluid

This is relatively new technique for SLN production and has the advantage of solvent – less processing. There are several variations in this platform technology for powder and nanoparticle preparation. SLN can be prepared by the rapid expansion of super critical carbon dioxide (RESS) method.

6. Spray drying method [16]

It is an alternative procedure for lyophilization in order to transform an aqueous SLN dispersion into a product. It's a cheaper method than lyophilization. This method particle aggregation due to high temperature, shear forces and partial melting of the particle. Frietus and mullera recommends the use of lipid with melting point > 70°C for spray drying. The best result was obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol – water mixtures.

7. Double emulsion method

For the preparation hydrophilic loaded SLN, a novel method based on solvent – evaporation has been used. Here the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase of w/o/w double emulsion.

No single synthesis technique has demonstrated exceedingly superior performance. Each technique possesses strengths and weaknesses.

TECHNIQUES	STRENGTHS	WEAKNESSES
High shear homogenization	Low capital cost Demonstrated at lab. Scale Reduced shear stress	Energy intensive process Biomolecule damage Polydisperse distribution
Ultrasonication		Potential metal contamination
High pressure homogenization	Scalable Mature technology Continous operation Commercially demonstrated No dilution solidification	Energy intensive process Unproven scalability Extremely energy intensive process Polydisperse distribution Biomolecule damage
Solvent evaporation	Monodisperse distribution Low mechanical energy input	Residual organic solvent Extremely sensitive change
Microemulsion		Labor intensive formulation work

Strengths and weaknesses of different formulation techniques

Drug incorporation models of SLN [17]

- Solid solution model
- Core shell model

Solid solution model

The drug is molecularly dispersed in the lipid matrix when the particles are produced by the cold homogenization technique and using no surfactant (or) no – drug solubilizing surfactant.

Core shell model

A solid lipid core forms when the recrystallization temperature of the lipid surrounding the drug as the membrane.

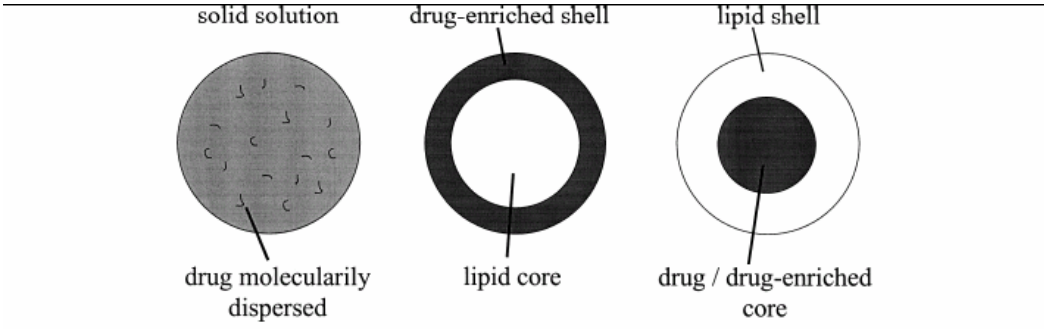


Figure – 5

Proposed structural models for drug loading profiles in lipid nanoparticles

Estimation of incorporated drug

Entrapment efficiency [16, 17, 27, 28]

Is the prime importance in SLN, since it influences the release characteristics. The amount of drug encapsulated per unit weight of nanoparticles is determined after separation of the free drug and solid lipids from the from the aqueous medium. This

separation can be carried out using ultracentrifugation, centrifugation filtration (or) gel permeation chromatography.

Centrifugation filtration [26]

Filters such as ultra free – MC (or) ultrasort – 10 are used along with classical centrifugation techniques. The degree of encapsulation can be assessed indirectly by determining the amount of drug remaining in supernatant after centrifugation filtration / ultracentrifugation of SLN suspension (or) alternatively by dissolution of the solvent and subsequent analysis.

Gel permeation chromatography [32, 33]

The unentrapped drug is removed by gel filtration of SLN dispersion through a sephadex G – 50 column and elution with phosphate buffered saline (or) normal saline.

Analytical characterization of SLN quality

An adequate characterization of the SLN's necessity for the control of the quality of the product.

a) Entrapment efficiency [27, 28, 29]

SLN dispersion is prepared, and it is determined for the entrapped drug by centrifugation filtration method. SLN dispersion is centrifuged in a refrigerated centrifuge and the supernatant is collected and filtered before analyzing the resultant solution by appropriate assay method for the drug.

$$\text{Entrapment efficiency} = \frac{\text{Wt. of drug incorporated}}{\text{Wt. of drug initially taken}} \times 100.$$

b) Particle size and zeta potential [30, 31]

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine measurements of particle size. This method covers a range to about 3 microns. PCS is a good tool to characterization of nanoparticles, but it is not able to detect larger microparticles. Electro microscopy provides, in contrast to PCS and LD, direct information on the particle shape.

Zeta potential (ZP) measurements allow predictions about the storage stability of colloidal dispersion.

c) Differential scanning calorimetry (DSC) [31]

DSC is the most commonly applied to assess the status of the lipid. DSC uses the fact that different lipid modifications possess different melting points and melting enthalpies.

In vitro drug release from SLN [23, 29, 58]

There are various methods used to study the in vitro release of the drug are:

- Side by side diffusion cells with artificial (or) biological membrane.
- Dialysis bag diffusion technique
- Reverse dialysis bag technique
- Agitation followed by ultracentrifugation (or) centrifugal ultra filtration

Dialysis tubing is the most commonly used method.

Dialysis tubing [29]

In vitro drug release could be achieved by using dialysis tubing. The SLN dispersion is placed in pre washed dialysis tubing which can be hermetically sealed. The dialysis sac then dialyzed against a suitable dissolution medium at suitable intervals.

Influence of ingredient composition on product quality

Influence of lipid [17, 27, 34]

Using the hot homogenization, it has been found that the average particle size of SLN increasing with higher melting lipids. SLNs have slower degradation because of their solid matrix. The influence of lipid composition of the particle size was also confirmed on SLN produced by high shear homogenization. Factors such as velocity of lipid crystallization, lipid hydrophobicity, influence of self emulsifying properties of lipid on the shape crystals.

Effect of emulsifier [34]

Emulsifiers have the impact on the quality of the SLN dispersion. SLN produced with lower concentration of the emulsifier leads to microparticles. High concentration of the emulsifier reduces the surface tension and facilitates the particle partition during homogenization. It could be shown that the degradation velocity increased with decreasing length of the fatty acid length when using glycerides as lipid matrix.

Fate of SLN after oral administration [35, 36, 37]

The oral route continues to be a challenge as well as the most attractive way to administer drugs because of its unquestionable commercial potential. Incorporation of drugs into lipid nanoparticles opens the perspective of enhanced and / or less variable

bioavailability and prolonged plasma levels. While these systems may provide the greatest flexibility in the modulation of the drug release profile GIT, they may also provide protection against chemical degradation for labile drug molecules.

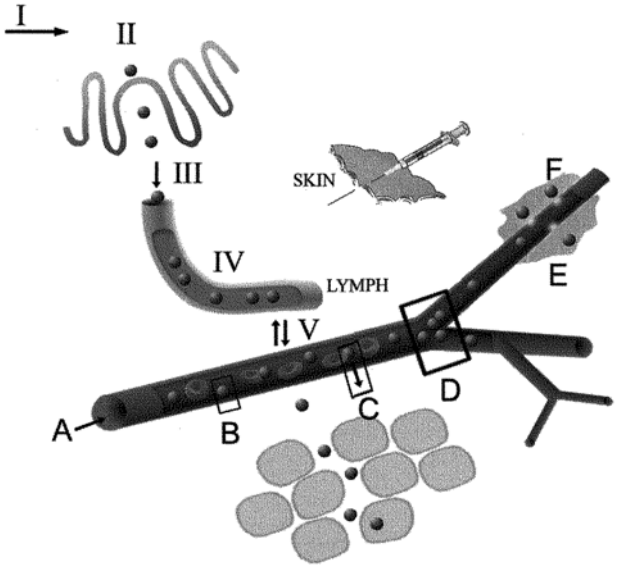


Figure – 6

A diagrammatic representation of some of the areas where flow and transport of nanoparticles is key. I: flow in the GI tract after oral administration; II: access to and adhesion to M-cells of Peyer 's patches or to enterocytes; III: passage into the mesenteric lymph; IV: flow in the lymph vessels and entrapment in the lymph nodes (not shown); V: transport between lymph and blood. A: blood flow; B: adhesion to capillary walls; C: extravasation and flow in tissue; D: flow and deposition at vessel bifurcations; and E: movement into tumours. Each route (the subcutaneous route is also indicated) will involve a complex sequence of nanoparticle pathways, most involving lymph, blood and intestinal fluid.

Routes of administration and their biodistribution

Various administration routes are:

Per oral administration [38, 39]

Yang et al, investigated camptothecin (CA) [39] containing SLN were produced from stearic acid 2%, lecithin 1.5%, and poloxamer 188 0.5% claimed encapsulation efficiency of CA was 99.6%. the conclusion from the study was that SLN are promising sustained release system of CA and other lipiphilic drugs after oral administration.

Parenteral administration [39]

Yang et al, reported on the pharmacokinetics and body distribution of canptothecin after IV injection in mice. Gasco et al, produced stealth and non stealth SLN and studied them in cultures of macrophages, and after loading them paclitaxel in vivo.

Transdermal application [40]

Liu et al, investigated triamcinolone acetonide acetate (TAA) containing SLN were produced from compritol 888 ATO (3.6%), soy lecithin (1.8%), Tween 80 (3.0%) and claimed encapsulation efficiency was 91.2%.

Cosmetic application [41, 42]

Li et al, studied penciclovir [44] loaded SLN composed of glyceryl monostearate, tween 80, poloxamer 188, brij 78 for percutaneous permeation and skin targeting.

Methotrexate [44] loaded SLN composed of cetyl alcohol, stearic acid, tween 80 was developed as topical gel to improve therapeutic index of methotrexate compared to topical formulations currently available in the treatment of psoriasis.

Sterilization of SLN [18]

For intravenous and ocular administration SLN must be sterile. The temperature reach during sterilization by autoclaving presumably causes a hot o/w micro emulsion and probably alters the size of the hot nanoparticles. Autoclaving at 121°C cannot be performed when using sterically stabilizing polymers, e.g. poloxamer series. This can be avoided by reducing the autoclaving temperature (e.g. 121°C to 110°C, but

simultaneously prolonging the autoclaving time). It can be concluded that SLN dispersions can also be sterilized by filtration similar to emulsions for parenteral nutrition. It is highly important to filter them in the liquid state; this allows even particles with a size larger than the pores in the filter to be filtered.

Advantages of SLNs [18, 20, 36]

- Control and / or target drug release
- Excellent biocompatibility
- Improve stability of pharmaceuticals
- High and enhanced drug content
- Easy to scale up and sterilize

Disadvantages of SLNs [17, 36]

- Particle growth
- Unpredictable gelation tendency
- Unexpected dynamics of polymorphic transitions

Application of SLN

SLN as gene vector carrier [30, 45, 46]

In one work the gene transfer was optimized by incorporation of the diametric HIV – 1 HAT peptide into SLN vector. Ther are several recent reports of SLN carrying / genetic / peptide materials such as DNA and peptide materials.

SLN as topical use [41, 42]

SLN and NLC have been used to for topical application for various drugs such as tropilide, imidazole, antifungals, vitamin – A, isotretinoin, ketaconazole, DNA, flurbiprofen and glucocorticoids. By using glyceryl behanate as lipid matrix vitamin – A loaded SLN was prepared.

SLN as cosmeceuticals [47]

The SLN have been applied in the application of sunscreens and as an active carrier agent for molecular sunscreens and UV blockers.

SLN for potential agriculture applications [48]

Essential oil extracted from Artemesia arboreseens L when incorporated into SLN, were able to reduce the rapid evaporation compared with emulsions and the systems have been used in agriculture as suitable carrier of safe pesticides.

SLN as targeted as carrier for anticancer drug to solid tumor [38, 44, 49]

SLN have been used as drug carriers. Tamoxifen, an anticancer drug incorporated in SLN to prolong the release of drug after IV administration in breast cancer. Tumor targeting has been achieved with SLN loaded drugs like methotrexate and camptothecin.

SLN in breast cancer and lymphnode and metastases [50]

Mitoxantrone SLN local injections were formulated to reduce the toxicity and improve the safety and bioavailability of the drug.

CHAPTER III

LITERATURE REVIEW

1. **Chattopadhyay et a1**., developed a lipid nanoparticle system for the enhanced brain delivery of the potent and frequently used HNP1, atazanavir.

The lipid nanoparticle was prepared by thin film hydration technique. The SLNs were analyzed particle size, encapsulation efficiency, morphology, zeta potential and drug release. Cell viability experiments demonstrated that SLNs exhibit no toxicity in HCMEC/D3 cell. Further more a release of rhodamine -123 by SLNs also resulted in a higher cell accumulation.

2. **Triplett II M.D et a1**., studied about SLN of β-carotene using statistical experimental design. Different formulation and high shear homogenization process effects on particle size, stability, drug release have been investigated . β-carotene was incorporated into stearic acid to investigate the effects of introducing a drug into the base SLN system. β-carotene entrapment efficiency average 40%. The statistical experimental design was found to be much broader that suggested in literature.

3. **Liu. W. et a1**., investigated SLN hydrogel for transdermal iontophoretic drug delivery. Triamcinolone acetonide was employed as model drug. SLN containing the drug was incorporated into carbopol and gel was prepared. The use of TAA-SLN carbopol gel as a vehicle for the transdermal iontophoretic drug delivery of TAA was evaluated in vitro using horizontal diffusion cell fitted with porcine ear skin. Transdermal penetration of TAA from TAA-SLN carbopol gel cross the skin tissue was significantly enhanced by iontophoresis.

4. **Ugazio.E. et a1**., prepared cyclosporine-A (CYA) solid lipid nanoparticles a potent immunosuppressive drug from o/w microemulsion method consisting of stearic acid, phosphotidylcholine and taurocholate. The claimed encapsulation efficiency was 13%.The SLN was characterized by entrapment efficiency, particle size, morphology and in vitro drug release. In vitro release of CYA form SLN is very controlled and it is proposed for most administration routes, in particular for the duodenal route.

5. **Abdelbary G. et a1**., studied the feasibility of the inclusion of a water insoluble drug diazepam (DZ) into solid lipid nanoparticles(SLN).This work also describes a new approach to prepare suppositories containing DZ-loaded SLN dispersions, as potential drug carrier for the rectal route. Modified high-shear homogenization and ultrasound techniques were employed to prepare SLN. The DZ loaded SLN was evaluated for entrapment efficiency, particle size, morphology and in vitro drug release. The results revealed that the SLN-based suppositories as final dosage form would provide means by which the DZ-loaded SLNs would be administered rectally. It appears that SLN and the prepared suppositories from SLN offer a promising delivery system for the prolongation of DZ release through the rectal route.

6. **Xu.Z. et a1**., designed and prepared a new docetaxel-loaded hepatoma targeted solid lipid nanoparticle (tSLNs) with galactosylated dioleoylphosphotidyl ethanol amine. The tSLNs were investigated for cellular cytotoxicity, cellular uptake, subcellular localization, in vivo toxicity, therapeutic effect, biodistribution and histology. The results showed that the cytotoxicity of tSLNs against hepato celluar carcinoma cell line BEL7402 was superior to taxotere® and non-targeted SLNs (nSLNs). The studies on cellular uptake and biodistribution indicated better autitumor

efficacy of tSLNs. These results implied that tSLNs could enhance its antitumor activity in VNO with low system toxicity.

7. **Yu et a1**., studied to enhance the liver targeting and reduce the side effects by synthesizing **N**1-stearyl-5-fu and incorporation into solid lipid nanoparticles (SLN). **N**1-stearyl-5-fu (5-Fus) was prepared by physical agglomeration method. The mean diameter of 5-Fus-SLN was 240.19nm and the drug loading was 20.53%.compared with 5-Fu injection,the distribution of 5-Fu-SLN in mice showed that 5-Fus-SLN could double 5-Fu concentration in mice livers. In conclusion, 5-Fus-SLN has significant liver targeting properties.

8. **Venkateswarlu et a1**., studied the enhancement of bioavailability of lovastatin by preparing solid lipid nanoparticles (SLN) of lovastatin using triglycerides by hot homogenization followed by ultrasanication. Bioavailabilities studies showed that the relative bioavailabilitied of lovastatin and lovastatin hydroxy acid of SLN were increased by ~173% and ~324% sespectively compared with lovastatin suspension, after intraduodenal administration to male wistar rats.

9. **Lu et a1**., prepared solid lipid nanoparticles of mitoxantrone for local injection against breast cancer and its lymph mode matastasas. Film dispersion-vitrasonication method was used to prepare mitoxantrone(MTO)-SLN. The claimed encapsulation efficiency was 87%. The in vitro release study revealed a profile of sustained release of MTO from MTO-SLN without burst effect. Human MCF-7 breast cancer in nude mice and animal model of P388 lymph node metastases in kunming mice was investigated for therapeutic effects. The percent inhibition of MTO-SLN against breast cancer was 81%.

10. **Asasutjarit et a1**., investigated mode equations describing the effect of solid lipid nanoparticles (SLN) formulation compositions on their size and zeta potential using face- centered central composite design. SLN was prepared using cetylpalmitate at varying concentrations of tween 80 and span 85 mixtures, dimethyldioctadecyl ammonium bromide (DDAB) and cholesterol. The ability of SLN to form complex with PH is-HIV-hugag was evaluated by electrophoretic mobility shift assay. In conclusion, the regression analysis showed that the model equations of responses fitted well with quadratic equations.

11. **Shafiq-un-nabi et a1**., prepared nanoemulsion of highly lipophilic (or) poorly water soluble drug to enhance the solubility and bioavailability of lipophilic drug. Ramipril was used as model drug.

12. **Jores et a1**., investigated the structure and performance of NLC's and SLN's. colloidal dispersion were produced by high-pressure homogenization and characterized by laser diffraction, photon correlation spectroscopy, wide angle x-ray scattering and differential scanning calorimetry. Nuclear magnetic resonance spectroscopy and electron spin resonance experiments performed to investigate NLC nanoparticles showed any advantage with respect to incorporation rate to the drug compared with conventional nanoemulsions.

13. **Murthy S.R. et a1**., studied the effect of lipid matrix on the entrapment of olanzapine (OL). OL loaded solid lipid nanoparticles was prepared using lipids like glyceryl monostearate (GMS), precirol ATO 5 (PRE), glyceryl tristearate (GTS), and witepsol E85(WE85) and poloxamer 407 and hydrogenated soya phosphotidylcholine as

stabilizers using a holt melt emulsification high pressure homogeniziation technique. The highest partition coefficient for OL with melted lipids and PH 7.4 phosphate buffer (PH 7.4 PB) was obtained with GTS. The entrapmrnt efficiency was in the following order. GTS SLNS> PRE SLNs> WE85 SLNs> GMS SLNs. Relase of OL from the SLNs was sustained up to 48 hours in PH 7.4 PB and obeyed higachi's release kinetics.

14. **Hou. D et a1.,** produced solid lipid nanoparticles (SLNs) using modified high shear homogenization and ultrasound techniques. Mifepristone had been used as model drug. Entrapment efficiency (EE%)of SLNs was more than 87 present and showed relatively long-term physical stability as the leakage was very small after being stored up for one month.

15. **Kuo Y.C. et a1**., fabricated cationic solid lipid nanoparticles (CSLNs) by microemulsion method. Polysorbate 80 were used as stabilizers,and the lipid phase contained cationic stearylamine (SA) and dioctadecyl ammonium bromide (DODAB) and non ionic compritol 888 ATO5 (CA) and cacoa butter (CB). Several factors such as entrapment efficiency, releases kinetics and the distribution of saquinavir (SQV) in (SLNs) were analyzed. The result indicate that the mixture of SA and DODAB and the mixture of CA and CB were beneficial to the entrapment efficiency of SQV.

16. **Attama.A.A.et.al.,** investigated the characterization of silid lipid nanodispersions (SLN) prepared with a 1:1 mixture of theobroma oil and goat fat and phospholipon 90G (P90G) as a stabilizer heterolipid and using polysorbate 80 as mobile surfactant. The SLN were characterized by time resolved particle size analysis, zeta potential and osmotic pressure measurements, differential scanning calorimetry and

wide angle X- ray diffraction (WAXD). Results indicate lipid mixtures produced SLN with lower crystallinity an higher particle sizes compared with SLN prepared with theobroma oil alone with or without P90G.

17. **Heiati.H.et.a1.,** prepared solid lipid nanoparticles with trilaurin(TL) and dipalmitoylphosphatidyl choline (DPPC) or a mixture of DPPC and dimyristoylphosphotidyl glycerol (DMPG) to produce SLNs. The in vitro release of AZT-P from different SLNs formulation was studied at 37°c using a bulk equilibrium reverse dialysis sac technique. Increased drug release was observed in SLNs formulated with phospholipids (PLS) having a transition temperature below 37°c.

18. **Paliwal.R.et a1**., prepared methotrexate SLN using stearic acid, glyceryl monostearate, tristearin, and compritol 888 ATO by solvent diffusion method to study the effect of lipid core material on characteristics of solid lipid nanoparticles designed for oral lymphatic delivery. The SLN were characterized for shaped, particle size, zeta potential and percentage drug content and its release. The comparative study conducted on MTX bearing SLN revealed that the formulation that the formulation based on compritol 888 ATO could improve the oral bioavailability of MTX.

19. **Rudolph et a1**., studied to optimize gene delivery of SLN-based gene vectors by incorporation of a dimeric HIV-1 TAT (peptide) TAT2 into SLN gene vector. In vitro studies revealed TAT2 into SLN gene vectors induce an increase of gene expression when compared with mutant TAT2-M1 and 4-8 times higher as compared with PEI.

20. **Bargoni et a1**., studies the uptake and transport of solid lipid nanoparticles (SLN) into the lymph and blood after duodenal administration in rats. Unlabelled and labeled SLN (average diameter of 80nm) were administered intraduodenally to rats. The biological sample were analyzed by photon correlation spectroscopy (PCS), transmission electron microscopy (TEM). TEM analysis evidenced SLN in lymph and blood after duodenal administration to rats.

21. **Jani et a1**., studied the oral absorption of fluorescent polystyrene nanospheres, of diameters ranging from 50nm to 3µm, administered orally to rats for 10days, and found that uptake and translocation were inversely size dependent.

22. **Huang G.et a1**., prepared temozolomide solid lipid nanoparticles (TMZ-SLNs) by emulsification and low temperature solidification method. In vitro drug release was conducted in phosphate buffered saline (PH 6.8) at 37°c. The results show that the TMZ-SLNs had an average diameter of 65.9±36mv and the in vitro drug release was monitored for up to 3 days. And the release behavior was in accordance with higuchi equation.

23. **Reddy et a1**., prepared solid lipid nanoparticles of etoposide using glyceryl monostearate (monoglyceride), glycerol distearate (diglyceride) and tripalmitin by slight modification of melt emulsification and homogenization technique. The melt was that heated to 5°c above the melting point of glycerides and emulsified using a blade-type stirrer at 2000rpm.

24. **Fang.Y. et a1**., developed solid lipid nanoparticles using precirol ATO 5. SLN showed mean particle size of 300nm. Tween 80 and soyabean lecithin and used as emulsifiers. Differential scanning calorimetries studied were performed to characterize the physiochemical properties of SLN.

25. **Helgason.T et a1**., studied the effect of surfactant and stability of tween 20 stabilized tripalmitin solid lipid nanoparticles (SLN). A lipid phase of 10% w/w and 2% tween 20 was used. The mean particle diameter of 134nm was observed. At low surfactant concentration (<1%w/w) the SLN formed gels, at higher surfactant concentration (>1%w/w) a homogenous dispersion was formed.

26. **Singh et a1**., investigated solid lipid nanoparticles of vitamin-A palmitate using high pressure homogenization technique. The nanoparticle dispersion was evaluated for various parameters such as particle size, in vitro drug release. The SLN showed a mean particle size of 350 nm. DSC studies revealed no drug – excipient incompatibility. In vitro drug release studies showed prolonged drug release up to 24 hours.

27. **Doijad et al**., studied the overall improvement in the efficacy and enhancement of therapeutic index. Cisplatin loaded solid lipid nanoparticles (SLN) was prepared using microemulsification method by stearic acid, soy lecithin and sodium glycolate. The formulations were characterized with respect to size, morphology, zeta potential, entrapment efficiency, in vitro drug release profile and stability. SLN were oval with a diameter ranging from 250 to 500nm. Entrapment efficiency was found to be 74.53%.

28. **Lai et a1**., investigated cubic nanoparticles of glyceryl monooleate (GMO)/poloxamer 407 as potential oval drug delivery system to enhance the bioavailability of water insoluble model drug simvastatin. The mean diameter of cubic nanoparticles varied within the range of 100-150nm. Entrapment efficiency over 98% was achieved due to high affinity of simvastatin to the hydrophobic regions of the cubic phase.

29. **Bhalekar et a1.,** prepared miconazole nitrate loaded solid lipid nanoparticles (MN-SLN) effective for topical delivery. Compritol 888 ATO as lipid, propylene glycon (PG) to increase solubility of the lipid, tween 80 and glyceryl monostearate were used as surfactants. SLN exhibited average size between 244 and 799nm. Entrapment efficiency ranging from 80% to 100%. The MN-SLN was characterized for particle size and entrapment efficiency.

30. **Luo.Y. et a1**., prepared vinpocetine loaded solid lipid nanoparticles using glyceryl monostearate. Ultrasonic solvent emulsification technique was used. SLNs were investigated for mean particle size, drug loading capacity, drug entrapment efficiency (EE), ε1 zeta potential. The drug release was preformed in 0.1m Hcl, distilled water and phosphate buffer saline PH 7.4 using the dialysis bag method. In vitro release study showed a prolonged release for up to 96 hours.

31. **Li.H.et a1**., designed queretin loaded solid lipid nanoparticles (QT-SLNs) to evaluate the potential of using (SLNs) as an oval delivery carrier for poorly soluble drug. QT-SLNs were prepared by emulsification ε1 low temperature solidification. The average drug entrapment efficiency, drug loading and zeta potential were characterized. In vitro drug release studies were performed by using dialysis bag diffusion technique.

The SLN dispersion is loaded into pre soaked dialysis bags and placed in conical flask. The conical flask was placed at 37°C at a rate of 100 rpm.

32. **Subedi.R.K. et a1**., prepared solid lipid nanoparticles loaded with doxorubicin by solvent emulsification diffusion method using glyceryl caprate as lipid corn. The mean particle size measured by photon correlation spectroscopy was 199nm. The entrapment efficiency and drug loading capacity were investigated. In vitro release studies were preformed by dialysis bag diffusion technique. SLN dispersion was transferred to the dialysis tube and then sealed tube was introduced into conical flask containing phosphate buffer saline PH 7.4 at 37±1°C and 50 strokes per minute.

33. **Jawahar.N. et a1**., designed poly (D,L-Lactide-co-glycolide) (PLGA) nanoparticles of ramipril by nanoprecipitation method using tribloere polymeric stabilizer (pluronic F-68). The particles were characterized for drug content, particle size, and particle morphology. In vitro release studies were performed by dialysis bag diffusion technique. The prepared nanoparticles were placed in the dialysis bag and immersed in 50ml of phosphate buffer saline (PBS) PH 7.4. The entire system was kept at 37°± 0.5°C with continuous magnetic stirring at 200 rpm. The amount of drug dissolved was determined with uv-spectrophotometry at 207nm.

34. **Wang et a1**., prepared total flavones of hippophae thamnoides (TFH) solid lipid nanoparticles by heating ultrasonic dispersion and lyodhilization. Using monostearin as lipid and poloxamer 188 as surfactant. SLN were characterized for mean for mean particle size, zeta potential and entrapment efficiency. The percentage of incorporated drug in the lipid matrix was determined by ultracentrifugation. In vitro release study was

performed within 48 hours after the preparation of SLN. The TFH SLN dispersion held a uniform particle size distribution of about 200 nm. TFH SLN has a good sustained release effect.

CHAPTER-IV

SCOPE OF WORK

Hypertension (or) high blood pressure is a medical condition where in the blood pressure is chronically elevated. Persistent hypertension is one of the risk factors for strokes, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure.

Anti hypertensive drugs act by reducing the cardiac output and / or reducing the total peripheral resistance, without correcting the cause. Anti hypertensive drugs may ultimately reduce BP in humans by more than one mechanism. Further, the hemodynamic alterations produced by a single parenteral dose of a given drug may differ from the effects resulting from its prolonged oral administration [59].

Ramipril, a potent anti hypertensive drug is almost completely converted to its active metabolite ramiprilat (a dicarboxylic acid) by hydrolytic cleavage of the ester group in the liver, which has about 6 times the angiotension – converting enzyme inhibitor activity of ramipril. Ramipril categorized as a class IV / II drug according to biopharmaceutical classification system (BCS) because of its low solubility and poor permeability. Ramipril is a highly lipophilic log P (octanol / water) 3.22, poorly water soluble drug with absolute bioavailability of 28 – 30% of variable oral absorption. The poor oral bioavailability is due to the poor / low solubility and poor permeability [52].

Ramipril undergoes significant ‘first pass metabolism’. The half life of ramipril and its metabolite is 2 and 18 hrs respectively.

Based on these, the aim this current study was to produce and characterize ramipril loaded solid lipid nanoparticles using various monoglycerides (Glyceryl monostearate and Glyceryl monooleate), non ionic surfactants (Tween 80, Span 20 and Poloxamer 188) as stabilizers by hot homogenization and ultrasound dispersion method.

Because solid lipid nanoparticles, an alternative colloidal carrier (transport) system having ability to improve the solubility / permeability of lipophilic drugs and enhance drug absorption and toxicity. SLN improve targeting and stability of drug.

Thus the objective of SLN as an alternative colloidal carrier system of ramipril was developed for improving the dissolution rate of ramipril that would increase the biological activities.

PLAN OF WORK

PART-I

1. Determination of λmax of Ramipril
2. Calibration curve for the drug in distilled water and phosphate buffer saline pH7.4

PART-II

1. Formulation of ramipril loaded solid lipid nanoparticles using different concentrations of non ionic surfactants (Tween 80, Poloxamer 188 and Span 20) and lipids (Glyceryl monostearate and Glyceryl monooleate) by hot homogenization and ultrasound dispersion method.

PART-III

Evaluation of Ramipril loaded solid lipid nanoparticles

1. Determination of entrapment efficiency
2. In –vitro release characteristics of SLN dispersion in phosphate buffer saline P^H 7.4

PART-IV

1. Measurement of particle size of ramipril loaded solid lipid nanoparticles.
2. Morphological studies of SLN dispersion using scanning electron microscopy

PART-V

1. Stability studies of SLN dispersion at refrigerated and room temperature.

PART-VI

1. Differential scanning calorimetry studies of selected formulations to determine the status of the drug and lipid.

2. IR studies to determine interaction between lipids and drug.

CHAPTER-V

MATERIALS AND EQUIPMENTS

MATERIALS USED

1. Drug- Ramipril	- Madras Pharma
2. Glyceryl monostearate	- Central drug house
3. Glyceryl monooleate	- Otto chemicals
4. Poloxamer 188	- Madras Pharma
5. Polysorbate 80	- Sisco research lab
6. Sorbiton monolaurate	- Loba chemie
7. Chloroform	- Rankem
8. Methanol	- Rankem
9. Sodium chloride	- Central drug house
10. Potassium dihydrogen ortho phosphate	- Nice chemicals
11. Disodium hydrogen ortho phosphate	- Qualigens
12. Dialysis memebrane 50 – LA 387	- Himedia

EQUIPMENTS USED

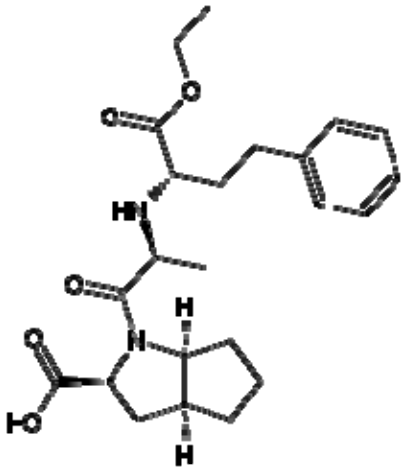
1. Rotary Flash Evaporator - Super fit rotary flash evaporator
2. Ultra Sonicator - Vibronic's Ultrasonic processor
3. Mechanical stirrer - Scientific industries
4. Electronic Balance - A&D Company, Japan
5. Magnetic Stirrer - MC Dalal & co
6. UV Visible Spectrophotometer - UV Pharma Spec 1700, Shimadzu
7. Refrigerator - Kelvinator

CHAPTER-VI

DRUG PROFILE

RAMIPRIL –DRUG PROFILE [51, 52, 53, 54, 60]

Structure



Chemical name

(2s, 3as, 6as) – 1 {(s) – N – [(s) – 1 – carboxy – 3 phenyl propyl] alanyl } octahydro cyclopenta (b) pyrrole - 2 – carboxylic acid, 1 – ethyl ester

Emperical Formula : $C_{23}H_{32}N_2O_5$

Description

Nature	:	white crystalline powder
Solubility	:	Freely soluble in methanol, slightly Soluble in water
Melting point	:	105°c and 112°c
Molecular weight	:	416.511gm/mol

Category

Angiotensin-converting Enzyme (ACE Inhibitors Systemic)

Identification

UV light absorption at 207 nm.

Pharmacodynamic properties

Ramipril an angiotensin-converting enzyme inhibitor. Ramipril an inactive prodrug is converted to active metabolite ramiprilat in liver. Ramiprilat the active metabolite competes with angiotensin converting enzyme blocking the conversion of angiotensin I to angiotensin II . It is a vasoconstrictor and a negative feedback mediator for renin activity. Lower concentrations result in decrease in blood pressure and an increase in plasma renin. Ramiprilat may also act on kininase II, an enzyme identical to ACE that degrades vasodilator bradykinin.

Pharmacokinetic properties

Absorption

- Extent of absorption in gastrointestinal tract is atleast 50 % to 60 %.
- T max is 1 hour for parent compound, 2 to 4 hour for metabolite.
- Plasma half life 2 to 4 hours.

Metabolism

- Ramipril is converted to active metabolite Ramiprilat in liver by the enzyme
- Ramiprilat has 6 times greater ACE inhibition activity than the parent compound.

Excretion

- 60 % of parent compound and metabolites are excreted in urine.
- 40 % of parent compound and metabolites are excreted in Faeces.
- Less than 2 % of unchanged drug excreted in urine.

Pharmacokinetic characters of Ramipril

- Oral Bioavailability : 28 % (Ramipril)
  44 % (Ramiprilat)
- Urinary excretion : Renal (60 %), Fecal (40 %).
- Plasma protein binding : 73 % (Ramipril)
  56 % (Ramiprilat)

Therapeutic indications

- Hypertension
- Congestive heart failure
- Myocardial Infarction
- To prevent stroke, Cardiovascular death.

Dose

- 5 to 10 mg per day.

Adverse Effects

- Postural Hypotension
- Hyperkalemia
- Cough
- Angio edema
- Neutropenia
- Agranulocytosis
- Anaphylactoid reactions
- Nausea
- Vomiting
- Dizziness

Drug Interactions

- Hyperkalemia with potassium sparing diuretics and potassium supplements.
- Antacids reduce bioavailability of Ramipril.
- Indomethacin(and other NSAIDS) attenuate the hyoptensive action.

Special Precautions

- Don't take potassium supplements without seeking medical advice
- Don't take during pregnancy.

Contra-indications

- Renovascular disease.

- Severe renal impairment.
- History of angio edema.
- During Pregnancy.
- Hypotension.

International Brand Names

Acovil (ES)
Altace (VE)
Cardace (ID, IN)
Corpril (TH)
Delix (DE)
Heartprilprotect (KP)
Hyperil (ID)
Hypren (AT)
Lostapres (AR)

CHAPTER-VII

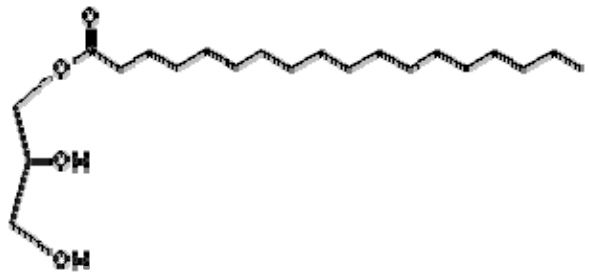
EXCIPIENTS PROFILE

GLYCERYL MONOSTEARATE [61]

SYNONYM

Glyceryl stearate, Monostearin

STRUCTURE



CHEMICAL NAME

3-Stearoyloxy-1,2-propanediol; Glyceryl stearate; Alpha-Monostearin; Monostearin; Octadecanoic acid, 2,3-dihydroxypropyl ester; Glycerin 1-monostearate; Glycerin 1-stearate; Glycerol alpha-monostearate; Glyceryl 1-monostearate; Stearic acid alpha-monoglyceride; Stearic acid 1-monoglyceride; 1-Glyceryl stearate; 1-Monostearin; 1-Monostearoylglycerol; 1,2,3-Propanetriol 1-octadecanoyl ester.

EMPIRICAL FORMULA

$CH_3 (CH_2)_{16}COOCH_2CHOHCH_2OH$

MOLECULAR WEIGHT

358.56

FUNCTIONAL CATEGORY

- Emulsifying agent

DESCRIPTION

White or cream colored waxy solid.

PROPERTIES

Physical state	:	white powder
Melting point	:	63 - 68 ^{o}C
Boiling point	:	> 100 ^{o}C
Solubility in water	:	soluble in hot water
Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	5.0

STABILITY AND STORAGE CONDITIONS

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

SAFETY

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS

Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust.

REGULATORY STATUS

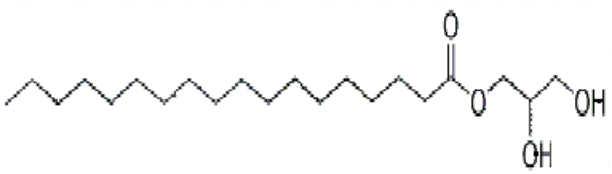
Induced in the FDA inactive ingredients. Recognized by GRAS status.

GLYCERYL MONOOLEATE [61, 62, 63]

SYNONYM

1 - Glyceryl monooleate, monoolein

STRUCTURE



CHEMICAL NAME

3-Stearoyloxy-1,2-propanediol; Glyceryl stearate; Alpha-Monostearin; Monostearin; Octadecanoic acid, 2,3-dihydroxypropyl ester; Glycerin 1-monostearate; Glycerin 1-stearate; Glycerol alpha-monostearate; Glyceryl 1-monostearate; Stearic acid alpha-monoglyceride; Stearic acid 1-monoglyceride; 1-Glyceryl stearate; 1-Monostearin; 1-Monostearoylglycerol; 1,2,3-Propanetriol 1-octadecanoyl ester.

EMPIRICAL FORMULA

$CH_3 (CH_2)_{16}COOCH_2CHOHCH_2OH$

MOLECULAR WEIGHT

358.56

FUNCTIONAL CATEGORY

- Emulsifying agent

DESCRIPTION

White or cream colored waxy solid.

PROPERTIES

Physical state	:	white powder
Melting point	:	63 - 68 ^{o}C
Boiling point	:	> 100 ^{o}C
Solubility in water	:	soluble in hot water
Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	5.0

STABILITY AND STORAGE CONDITIONS

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

SAFETY

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS

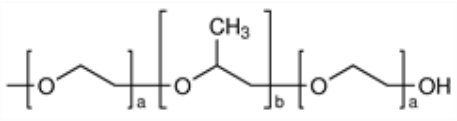
Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust.

POLOXAMER 188 [61, 64]

SYNONYM

Lutrol F 68, Pluronic F 68

STRUCTURE



CHEMICAL NAME

Polyethylene-Polypropylene Glycol

EMPIRICAL FORMULA

$HO(C_2H_4O)a(C_3H_6O)b(C_2H_4O)aH$

MOLECULAR WEIGHT

8400.00

FUNCTIONAL CATEGORY

- Emulsifying agent
- Sensitize drug resistant cancers to chemotherapy

DESCRIPTION

White to off white granules

PROPERTIES

Physical state	:	white powder
Solubility in water	:	soluble in water
Solvent solubility	:	soluble in methanol and chloroform mixture
HLB value	:	29.0

BIOLOGICAL EFFECTS OF POLOXAMER

Originally thought to be inert carrier molecules work led by Kabanov has recently shown that some of these polymers have a very real effect on biological systems independently of the drug they are transporting. The poloxamers have been shown to incorporate into cellular membranes affecting the microviscosity of the membranes.

STABILITY AND STORAGE CONDITIONS

It is stable under ordinary conditions, and should be stored in a well-closed container and protected from light.

SAFETY

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS

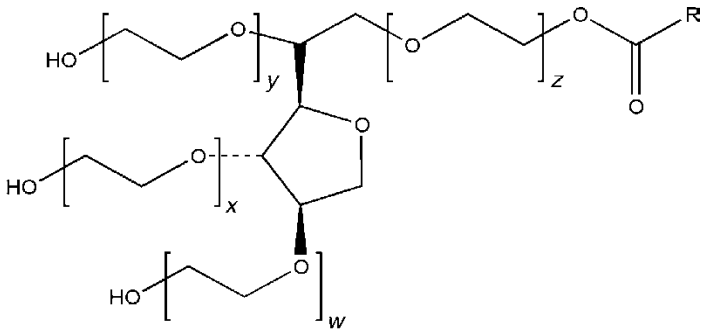
Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust.

POLYSORBATE 80[65]

SYNONYM

Atlas E, Cappmul POE-O, Glycospere o-20,Tego SMO 80,Tego SMO 80 X, Tween 80.

STRUCTURE



CHEMICAL NAME

(Z) Sorbitan mono-9- Octadecanoate poly (oxy 1,2, ethanediyl) derivatives.

EMPIRICAL FORMULA

$C_{64}\ H_{124}\ O_{26}$

MOLECULAR WEIGHT

1310.00

DESCRIPTION

Yellow oily liquid.

METHOD OF MANUFACTURE

Polysorbates are prepared from sorbitol in a three-step process. Water is initially removed from the sorbitol to form a sorbitan (a cyclic sorbital anhydride). The sorbitan is then partially esterified with a fattyacid such as oleic acid (or) stearic acid to yield a hexiton ester. Finally, ethylene oxide is chemically added in the presence of a catalyst to yield the polysorbates

PROPERTIES

Acid value – 2.0

Hydroxyl value – 65 – 80

Saponification value – 45 - 55

Density (g/cm^3) – $1.08 g/cm^3$

HLB Value – 15

Solubility – Soluble in ethanol and water. Insoluble in Mineral oil and vegetable oil.

FUNCTIONAL CATEGORY

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / Suspending agent.

STABILITY

- Gradual soap formation occurs with strong acids or bases

- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

- Daily intake according to the WHO limit is about 25mg/Kg body weight
- LD_{50} (Mouse, oral)-25g/Kg.

HANDLING PRECAUTIONS:

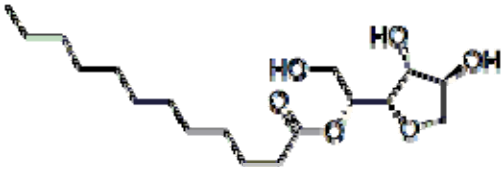
Eye protection and Gloves are recommended.

SORBITAN MONOLAURATE [66, 67]

SYNONYMS

Arlacal 20, Span 20, Sorbitan monolaurate.

STRUCTURE



CHEMICAL NAME

Sorbitan monododecanoate

EMPIRICAL FORMULA

$C_{18}H_{34}O_6$

MOLECULAR WEIGHT

346.46

DESCRIPTION

It occurs as yellow viscous liquid with a distinctive odour and taste.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan(1,4 Sorbitan) which is then esterified with the desired fatty acid.

FUNCTIONAL CATEGORY:

- Emulsifying agent.
- Non ionic Surfactant.
- Solubilizing agent.
- Wetting agent.

PROPERTIES

Acid value	:	3 to 7
Hydroxyl value	:	270 to 303
Iodine value	:	≤ 1
Density (g/cm^3)	:	1.032 gm/ml
HLB Value	:	8.6
Melting point	:	43^0C - 48^oC
Solubility	:	Soluble in oils and in most organic solvents. Insouble but dispersible in water.

STABILITY

- Gradual soap formation occurs with strong acids or bases.
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

CHAPTER-VIII

EXPERIMENTAL PROTOCOL

STANDARD CURVE FOR RAMIPRIL

PREPARATION OF CALIBRATION MEDIUM [68]

Phosphate buffer saline P^H 7.4

Dissolve 1.6 gm of sodium chloride, 0.038 gm of potassium dihydrogen phosphate, 0.472 gm of disodium hydrogen phosphate in specified quantity of distilled water and this solution is placed in 200ml volumetric flask and make up to the volume with distilled water.

PREPERATION OF STANDARD CURVE FOR RAMIPRIL [29]

The standard stock solution of ramipril is prepared by dissolving a known amount of drug in methanol and diluted with distilled water. From the above stock solution, drug having different concentration of 5, 10, 15, 20 … 50µg /ml is prepared in distilled water.

The resulting solution is scanned in UV spectrophotometer to find the λmax and the absorbance is measured at λmax (207nm). Taking concentration in X-axis and absorbance in Y-axis plots the standard curve.

The standard curve is prepared similarly in phosphate buffer saline P^H 7.4. The standard curve is used to estimate drug content, entrapment efficiency and percentage drug release.

FORMULATION OF RAMIPRIL LOADED SOLID LIPID NANOPARTICLES [5, 20, 21, 31, 69, 70]

Solid lipid nanoparticles are prepared by a hot homogenization followed by ultrasonication method. Ramipril loaded solid lipid nanoparticles is prepared by using two different type of lipids (monoglycerides) glyceryl monostearate and glyceryl monooleate (6% W/V) and various surfactants tween 80, poloxamer 188 and span 20 (1.0%, 1.5% and 2.0% W/V) are used as stabilizers to enhance the solubility of the drug in the lipid.

The drug concentration is kept as constant for each formulation (1mg/ml).the formulations are represented in Table 1.

PREPARATION OF RAMIPRIL LOADED SOLID LOADED NANOPARTICLES [20, 21, 31, 69, 70**]**

Ramipril loaded SLN is prepared by a hot homogenization followed by ultrasonication method. Ramipril (0.16% W/V to the lipid), monoglyceride (6% wt/vol) is dissolved in a 10 ml mixture of methanol and chloroform (1:1). Organic solvents are completely removed using a rotary flash evaporator. The embedded lipid layer was melted by heating 5°C above the melting point of the lipid. An aqueous phase is prepared by dissolving Tween 80 or Poloxamer 188 or Span 20 (1.0% or 1.5% or 2.0% W/V) in distilled water (sufficient to produce 30 ml of preparation) and heating to the same temperature of the oil phase. The hot aqueous phase is added to the oil phase and homogenization is performed (at 2500 rpm and 70°C) using a mechanical stirrer for 30 minutes. The coarse oil in water emulsion so obtained is ultrasonicated using a vibronic's ultrasonic processor for 25 minutes. Ramipril loaded SLN is finally obtained

by allowing the hot nanoemulsion to cool to room temperature and stored at 4°C in refrigerator.

EVALUATION OF RAMIPRIL LOADED SLN [23, 29, 31, 38, 46, 69, 70, 71, 72, 73]

The formulated ramipril loaded solid lipid nanoparticles are evaluated for its entrapment efficiency, drug content and in vitro drug release studies in phosphate buffer saline P^H 7.4.

Determination of entrapment efficiency [29, 31, 38]

Entrapment efficiency of SLN dispersion is determined by centrifugation filtration method. SLN dispersion containing 5mg of drug is placed in a refrigerated centrifuge and centrifuged at 20000 rpm for 1 hour and the supernatant is collected and filtered. The filtered solution is made up to desired volume with fresh phosphate buffer saline pH 7.4. The amount of drug that is not incorporated in the SLNs could be obtained by the UV – detection absorption percent.

The absorbance of the samples is measured at 207nm to estimate the percentage entrapment efficiency.

The entrapment efficiency is calculated by following formula:

$$\text{Entrapment efficiency} = \frac{\text{Wt. of drug incorporated}}{\text{Wt. of drug initially taken}} \times 100.$$

In vitro drug release [29, 69, 72]

The in vitro release of ramipril from different SLN was determined using dialysis bag diffusion technique. An accurately weighed amount of ramipril loaded SLN

dispersions equivalent to 2.5 mg ramipril is transferred to dialysis bag and sealed. The sealed bag is then suspended in a beaker containing 250ml of phosphate buffer saline pH 7.4 and it was allowed to rotate at a constant speed (50 rpm) at 37°C ± 0.5°C. Aliquots are withdrawn at predetermined intervals from the receptor compartment up to 12 hours and the same is replaced with fresh buffer and then the drug content is determined spectrophotometrically by measuring the absorbance at 207nm using respective receptor medium as blank to calculate the amount of drug released fro nanoparticles.

Particle size analysis [23, 46, 73]

The mean diameter of SLNs in the dispersion is determined by photon correlation spectroscopy (PCS) at a fixed angle of 90° at 25°C. Particle size is determined by using blue wave (microtrek) using disposable sizing cuvette. Before measurement 1 drop of sample is taken from each selected formulated nanosuspension and diluted in 10 ml of the dispersion medium (distilled water).

Scanning electron microscopy [71]

Average particle size and surface morphology of SLN are evaluated using scanning electron microscopy. A brass specimen stub is used for mounting the sample, and the wet sample is dried in room temperature and then coated with gold under vacuum for 60 seconds**.** Then the sample is observed under scanning electron microscope.

FT - IR studies [29, 70]

The interaction between the lipids with drug is studied by using IR Spectrophotometer. Drug, lipid and selected formulations are subjected to FTIR studies.

Stability studies [31, 38, 74]

All formulations of ramipril loaded SLN are subjected to stability studies. They are stored in two different temperatures, 4^0C and 25 ± 2^0 C and the entrapment efficiency is estimated for every 15 days.

CHAPTER-IX

RESULTS AND DISCUSSION

STANDARD CURVE OF RAMIPRIL [29, 68]

The λ max of ramipril was determined by scanning the 10μg/ml of the drug solution in distilled water and phosphate buffer saline P^H 7.4. It showed the λ max of 207nm in both distilled water and phosphate buffer saline P^H 7.4.

Linear correlation coefficient was obtained for calibration of ramipril in each medium. Ramipril obeys the beer's law within the concentration range of 5 to 50 μg/ml. Calibration plots of ramipril in both medium was shown in figure 7, 7a and 8. The λ max of ramipril is showed in UV graph.

FORMULATION OF RAMIPRIL LOADED SOLID LIPID NANOPARTICLES

Ramipril loaded solid lipid nanoparticles is prepared by using two different type of lipids (monoglycerides) glyceryl monostearate and glyceryl monooleate (6% W/V) and various surfactants tween 80, poloxamer 188 and span 20 (1.0%, 1.5% and 2.0% W/V) are used as stabilizers to enhance the solubility of the drug in the lipid. The prepared SLN dispersion was found to be uniform and homogenous in appearance.

ENTRAPMENT EFFICIENCY

In order to attain optimal encapsulation efficiency, several factors were varied including the type and concentration of the lipid and surfactant material used. In all the formulations, the impact of lipid and surfactant concentration was significant this was shown in table no: 4 and Figure 9.

Effect of surfactants on entrapment efficiency

For all SLN formulations prepared with Poloxamer 188 as stabilizer shows higher entrapment efficiency, the increase in the Poloxamer 188 concentration increased the entrapment as it was acting as a steric stabilizer (F4, F5, F6, F13, F14, F15). While the formulations (F1, F2, F3, F10, F11, F12) containing glyceryl monostearate and glyceryl monooleate as lipid material respectively, the increase in the entrapment upon increasing tween 80 concentration could be attributed to the added emulsification and stabilization effect of this lipid material in the presence of tween 80 [23]. This result was agreeing with the results obtained by **Abdelbary et al.,** Formulations containing span 20 as surfactants show a lower entrapment efficiency comparatively to other surfactants this could be due to the lower HLB value of Span 20. This result was agreeing with the results obtained by **Q. Lv et al [49].**

HLB values of non ionic surfactants are Pluronic F68 (29.0), Tween 80 (15.0), Span 20 (8.6).

Hence the entrapment efficiency of various SLN stabilized with different nonionic surfactants decrease in the order of

Pluronic F68 > Tween 80 > span 20

These results explained that the entrapment efficiency increases with increasing concentration of non ionic surfactants based on their hydrophilicity. **Abdelbary et al** developed the diazepam loaded solid lipid nanoparticles had the highest entrapment efficiency with poloxamer 188 than Tween 80 is consistent with these results [23].

Effect of lipid material on entrapment efficiency

It could be noted that for formulations prepared using either glyceryl monostearate or glyceryl monooleate as lipid matrix, there is a considerable difference

in the entrapment efficiency. For SLN formulations prepared with glyceryl monostearate (F1 –F9) and glyceryl monooleate (F10 – F18) as lipid matrix there is a noticeable influence on the entrapment efficiencies of prepared SLNs. This could be attributed to the difference in the solubility of the drug in the lipid **(Muller et al 2000)**. The formulations prepared with glyceryl monostearate (F1 – F3) using tween 80 and formulations F4 and F5 using poloxamer 188 as stabilizer shows higher entrapment efficiency when compared to glyceryl monooleate. But for formulations F6 – F9 shows lower entrapment efficiency comparatively to formulation prepared with glyceryl monooleate (F15 – F18), this may be due to the crystallization of the lipid phase, which produces a partial expulsion of the drug on the particle surface.

The order of entrapment efficiency of GMS SLN with non ionic surfactants was

1. Poloxamer 188 (PF - 68)

85.70% [GMS 6.0% + PF – 68 2.0%] > 84.13% [GMS 6.0% + PF – 68 1.5%] > 81.97% [GMS 6.0% + PF – 68 1.0%]

2. Tween 80 (T - 80)

81.22% [GMS 6.0% + T - 80 2.0%] > 80.06% [GMS 6.0% + T - 80 1.5%] > 78.72% [GMS 6.0% + T - 80 1.0%]

3. Span 20 (S - 20)

76.64% [GMS 6.0% + S - 20 2.0%] >74.85[GMS 6.0% + S - 20 1.5%] > 72.50% [GMS 6.0% + S - 20 1.0%]

The order of entrapment efficiency of GMO SLN with non ionic surfactants was

1. Poloxamer 188 (PF - 68)

86.40% [GMO 6.0% + PF – 68 1.0%] > 84.48% [GMO 6.0% + PF – 68 1.5%] > 79.82% [GMO 6.0% + PF – 68 2.0%]

2. Tween 80 (T - 80)

77.59% [GMO 6.0% + T - 80 2.0%] > 76.62% [GMO 6.0% + T - 80 1.5%] > 75.24% [GMO 6.0% + T - 80 1.0%]

3. Span 20 (S - 20)

82.71% [GMO 6.0% + S - 20 2.0%] >79.34% [GMO 6.0% + S - 20 1.5%] > 74.97% [GMO 6.0% + S - 20 1.0%] (Table - 4).

The entrapment efficiency of the formulations (F16 – F18) prepared with GMO using span 20 as stabilizer shows higher percentage entrapment efficiency comparatively than formulations (F7 – F9) prepared with GMS using span 20 as stabilizer. This could be attributed to the melting point of the lipid; GMO has a lower melting point (32.5°C) when compared to GMS (56°C) **(Zur muhlen et al.,)**.

IN VITRO RELEASE STUDIES

The in vitro drug release studies of ramipril loaded solid lipid nanoparticles was done using dialysis bag diffusion technique in phosphate buffer saline P^H 7.4.

In vitro release from SLNs

In order to compare the drug release profile from the prepared SLN formulations, cumulative percentage drug release at 2 hours (Q_{2h}) and cumulative percentage drug release at 12 hours (Q_{12h}) were analyzed [23].

Generally, all SLN formulation showed significant slower release than ramipril solution (1mg/ml). This confirms that a sink condition for ramipril release was accomplished and the dialysis bag used in the dissolution procedure does not limit ramipril release.

For formulations F2, F3, F4, F5, F6, F8, F9, F10, F11, F12, F14, F15 and F18 a rapid release was observed at the initial 2 hours and released nearly 30% of the drug was released and at the end of 12 hours 54 – 81% of drug was released from the SLN, this is shown in the table 5 – 7 and in figures 9 - 16. After that a prolonged release was obtained due to the slow diffusion of the lipophilic drug from the lipid matrix **(Abdelbary et al.,)**. The rapid release observed could be due to the drug enriched shell around the particles. Although the release rate of the SLN's could be influenced by complex factors, it was reported that among the factors that contribute to the fast release are the large surface area, a high diffusion coefficient, low viscosity of the matrix, and a short diffusion distance for the drug (release from the outer surface region of the nanoparticle).

Influence of lipid on drug release profiles of ramipril loaded SLN

By comparing the in vitro release results of SLN formulations prepared by GMS and GMO (F2 and F11, F3 and F12, F4 and F13, F5 and F14, F6 and F15, F8 and F17, F9 and F18) respectively. Formulations containing GMS as lipid matrix exhibited higher drug release extents than the formulations containing GMO as lipid matrix which shows a more sustained release. (Table – 5 - 7).

As mentioned above, GMO produces less ordered lipid crystals than GMS, leading to lower drug expulsion from the imperfect lattice, contributing to the prolonged release of the lipophilic drug **(Fahmy et al.,)**.

Moreover, GMO a lipid with lower melting point when compared to GMS. **Zur Muhlen et al.,** reported that lower melting point lipid can produce a controlled release from SLN. This is due to the presence of solid solution throughout the particle combined with the slow diffusion of drug from the lipid matrix.

Influence of surfactants on drug release profiles of ramipril loaded SLN

The results revealed that all the formulation made from GMS as lipid matrix and from surfactants (F3 - Tween 80 2.0%, F6 - Poloxamer 188 2.0%, F9 - Span 20 2.0%) as stabilizer showed a higher drug release from ramipril loaded SLN, this showed that the increase in the concentration of the surfactant there is an increased the drug release from the SLN. This is confirmed by **abdelbary et al.,[23].**

But, this difference could not be attributed to the formulations [F12, F15 and F18] made from GMO using Tween 80 2.0%, Poloxamer 188 2.0% and Span 20 2.0% respectively. These formulations showed a decrease in the drug release on the increase of concentration of surfactant, this may be due to lower melting point of GMO than GMS.

These results revealed that higher concentration of Poloxamer 188 2.0% [F6] exhibit higher drug release, **(Jawahar et al.,)** while using GMS as lipid matrix. In all the formulations prepared by using both lipids (GMS and GMO) with Span 20 as surfactants shows higher retarded drug release. This may be due to the lower HLB value of Span 20 (8.6) than the other surfactants used as stabilizers **Q. Lv et al [49]**.

Comparison of In vitro drug release of ramipril loaded SLN with ramipril pure drug solution

The release of ramipril from SLN is much slower and controlled than the ramipril pure drug solution (1mg/ml). Ramipril showed a release of about 77% within 4 hours. The sustained release profile of SLN was compared with pure drug solution and shown in table – 8 and figure - 18.

PARTICLE SIZE ANALYSIS

The mean particle size and its distribution were measured by dynamic light scattering technique. Six formulations were selected, three each from two lipids (GMS – F3, F6, F9) and (GMO – F12, F15, F18) containing Tween 80, Poloxamer 188 and Span 20 of similar concentration (2.0%) respectively.

Sizes of ramipril SLN of GMS and GMO with different non ionic surfactants (Tween 80, Poloxamer 188 and Span 20) were found to be in the range of 104 – 334 nm. The results are shown in table 9 and figure 18. The particle size distribution are shown in figure 19 – 22.

Effect of different lipids on the particle size of ramipril SLN

Figure 18 shows the effect of different surfactants on the particle size distribution of ramipril SLN made of GMS and GMO. There is a significant difference in the size of the particles with change in lipids. Ramipril loaded SLN with GMS as lipid matrix results in larger particle size comparatively to Ramipril loaded SLN with GMO as lipid matrix for all type of surfactants. This phenomenon could be explained by melting point of the lipid, GMS has a higher melting point than GMO, which results in

slower lipid recrystallization from the hot homogenized condition resulting in increase in particle size **(Zur muhlen et al.,)**.

Effect of different surfactants on the particle size of ramipril SLN

Figure shows the effect of different surfactants on the particle size distribution of ramipril SLN made of Tween 80, Poloxamer 188 and Span 20.

There is a significant difference in the size of the particles with the change in the type of the surfactants. SLN dispersion prepared using Poloxamer 188 (2.0%) [F6 and F15] shows lower particle size than the other surfactants. This result could be attributed due to the higher molecular weight of Poloxamer 188 and higher HLB value when compared to Tween 80 and Span 20 **(Muller et al.,)**.

Hence the particle size of various SLN stabilized with different nonionic surfactants increase in the order of

Poloxamer 188 > Tween 80 > span 20

SCANNING ELECTRON MICROSCOPY (SEM)

The particle size, shape and surface morphology was evaluated using SEM. SEM photographs of ramipril loaded SLN of selected formulations [F3, F6, F9, F12, F15 and F18] was observed. In all the selected formulations, the particles were almost spherical and homogenous. The micrographs also confirmed that SLNs was less than 400nm in size. The results showed that the ramipril loaded SLN particles have a spherical shape with smooth surface. This is shown in the figure 23 – 28.

FT - IR STUDIES

I.R studies were carried out to confirm the compatibility between the selected lipid, drug ramipril and SLN. The spectra obtained from the I.R studies are from 4400 cm^{-1} to 450 cm^{-1}. It was confirmed that there are no major shifting as well as no loss of functional peaks between the spectra of drug, lipid and drug loaded SLN (1743.98 cm^{-1}, 1654.04 $cm^{-1,}$ 1458.18 cm^{-1}, 2931.34 cm^{-1}).

STABILITY OF RAMIPRIL LOADED SLN

The stability studies of all formulations of ramipril loaded SLN were carried out by storing at 4^0C (refrigeration temperature) and $25^0C \pm 2^0C$ [38].

The entrapment efficiency of the drug in the SLN dispersion was estimated immediately after the preparation and the results are shown in the table. The entrapment efficiency of the SLN dispersion, after every 15 days was estimated and the results are shown in the table – 10 and 11.

The results showed that the entrapment efficiency of all formulations showed only a slight decrease after 30 days. Hence increase in temperature and storage period decreases the entrapment efficiency of SLN dispersion irrespective of the lipids and emulsifiers used for formulations. The results showed that the entrapment efficiency of the SLN dispersion stored in 4°C was more when compared to SLN dispersion stored in $25^0C \pm 2^0C$. Hence increase in temperature and storage period decreases the entrapment efficiency of SLN dispersion. Comparatively slightly high entrapment efficiency of drug was observed with SLN stored in refrigerated temperature.

CHAPTER X

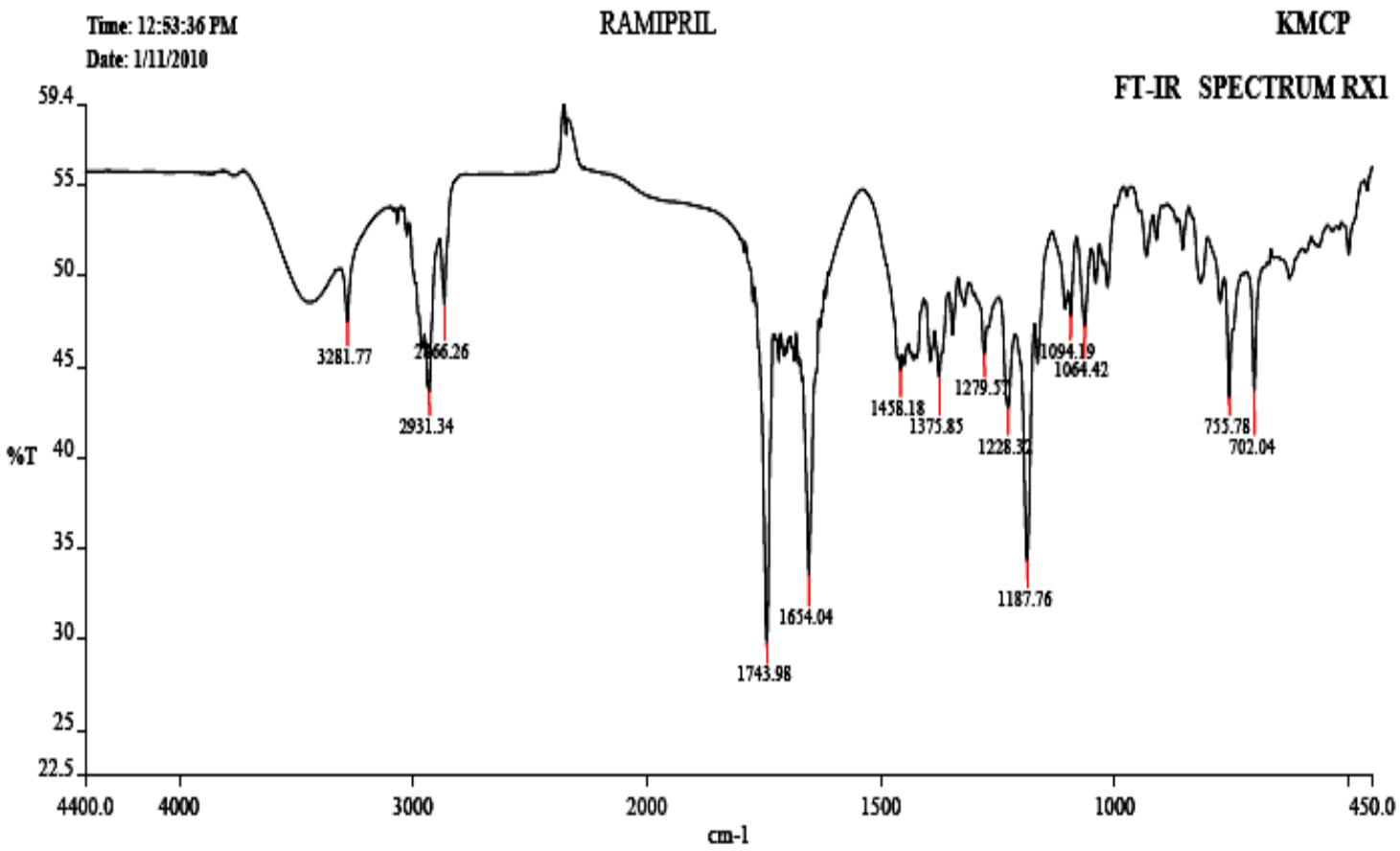
SUMMARY AND CONCLUSION

- The purpose of this research was to prepare ramipril loaded solid lipid nanoparticles for controlled release of drug and a trial to improve the bioavailability.
- Hot homogenization and ultrasound dispersion were employed to produce SLNs using biodegradable lipids and non ionic surfactants.
- The formulated SLNs were characterized for entrapment efficiency, particle size and in vitro release studies in phosphate buffer saline P^H 7.4.
- The nanoparticle colloidal drug delivery system of ramipril prepared type of lipids and non ionic surfactants obtained better entrapment efficiency.
- The better entrapment efficiency of SLNs was obtained with more hydrophilic surfactants (poloxamer 188) about 85.36% due to the higher HLB value of the surfactant.
- The results revealed that the increase in the surfactant concentration increases the entrapment efficiency for all formulations and the percentage entrapment efficiency of various non ionic surfactants was observed in the order of Poloxamer 188 > Tween 80 > Span 20.
- The particle size of the formulated ramipril SLNs exhibited nanometer size range spherical shape particles.
- The in vitro release studies revealed that the SLN formulations showed a prolonged drug release.
- SEM analysis of the SLN dispersion showed the spherical shape of the nanoparticles.
- Stability studies indicated that the entrapment efficiency of the SLN was not affected significantly in the refrigerated storage temperature. However there may be a

slight reduction in the entrapment efficiency of the SLN due to the drug expulsion from the crystal lattice.

- The results of the IR studies proved that no interactions between the drug, lipid and formulations.

It is concluded that the hot homogenization and ultrasound dispersion method, is a useful method for the successful incorporation of poorly water soluble drug ramipril with high entrapment efficiency. The prolonged release of the drug from the solid lipid nanoparticles suggests that the frequency of administration may be reduced. Further it may be presumed that if the nanometer range particles are obtained, the bioavailability may be increased. Hence we can conclude that solid lipid nanoparticles provide controlled release of drug and these systems are used as drug carriers for lipophilic drugs to enhance the bioavailability of poorly water soluble drugs through nanoparticle as a drug delivery system.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KMCP
FT-IR SPECTRUM RX1
%T
59.4
55
50
45
40
35
30
25
22.5
4400.0
4000
3000
2000
1500
1000
450.0
cm-1
3281.77
2931.34
1743.98
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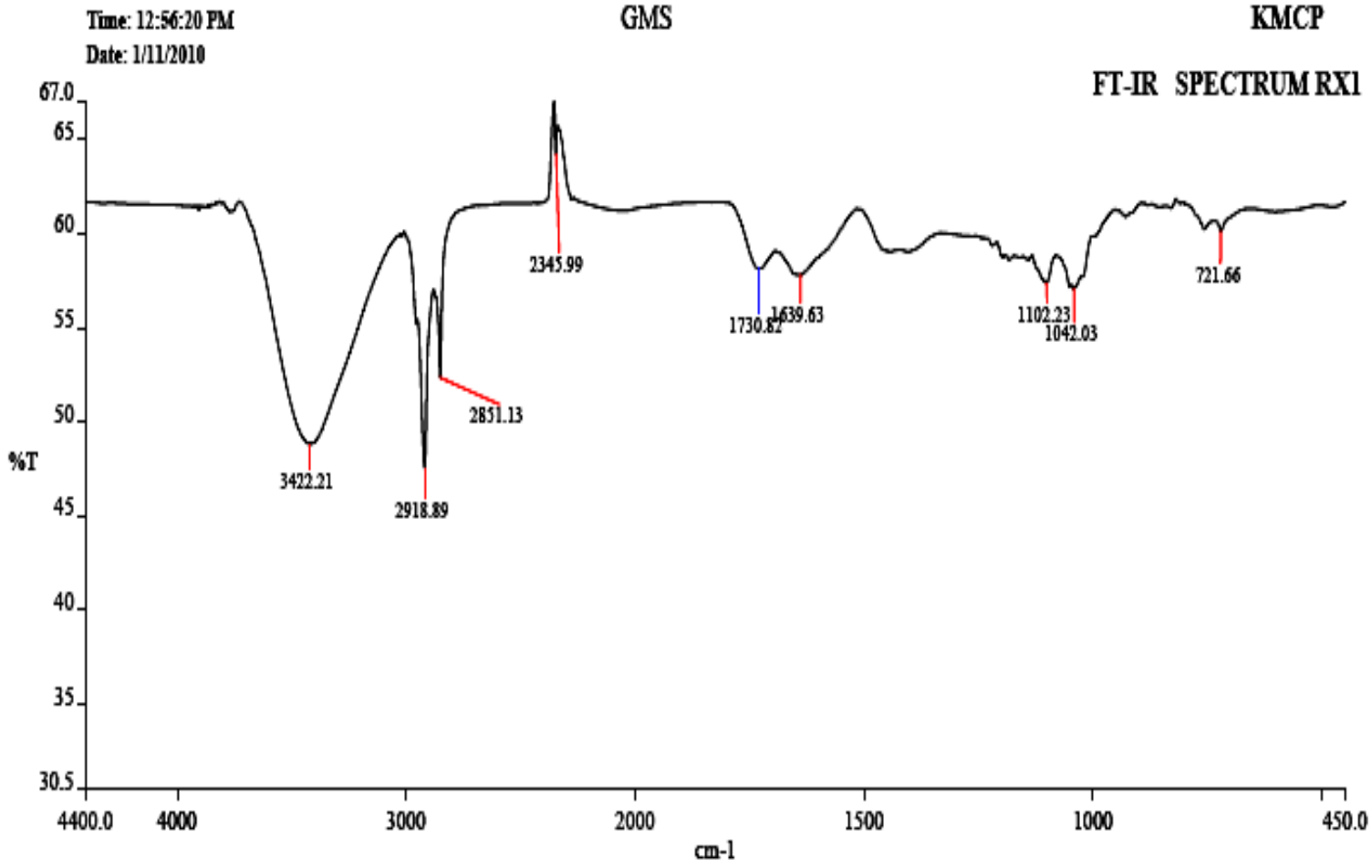
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45
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35
30.5
%T
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2918.89
2851.13
2345.99
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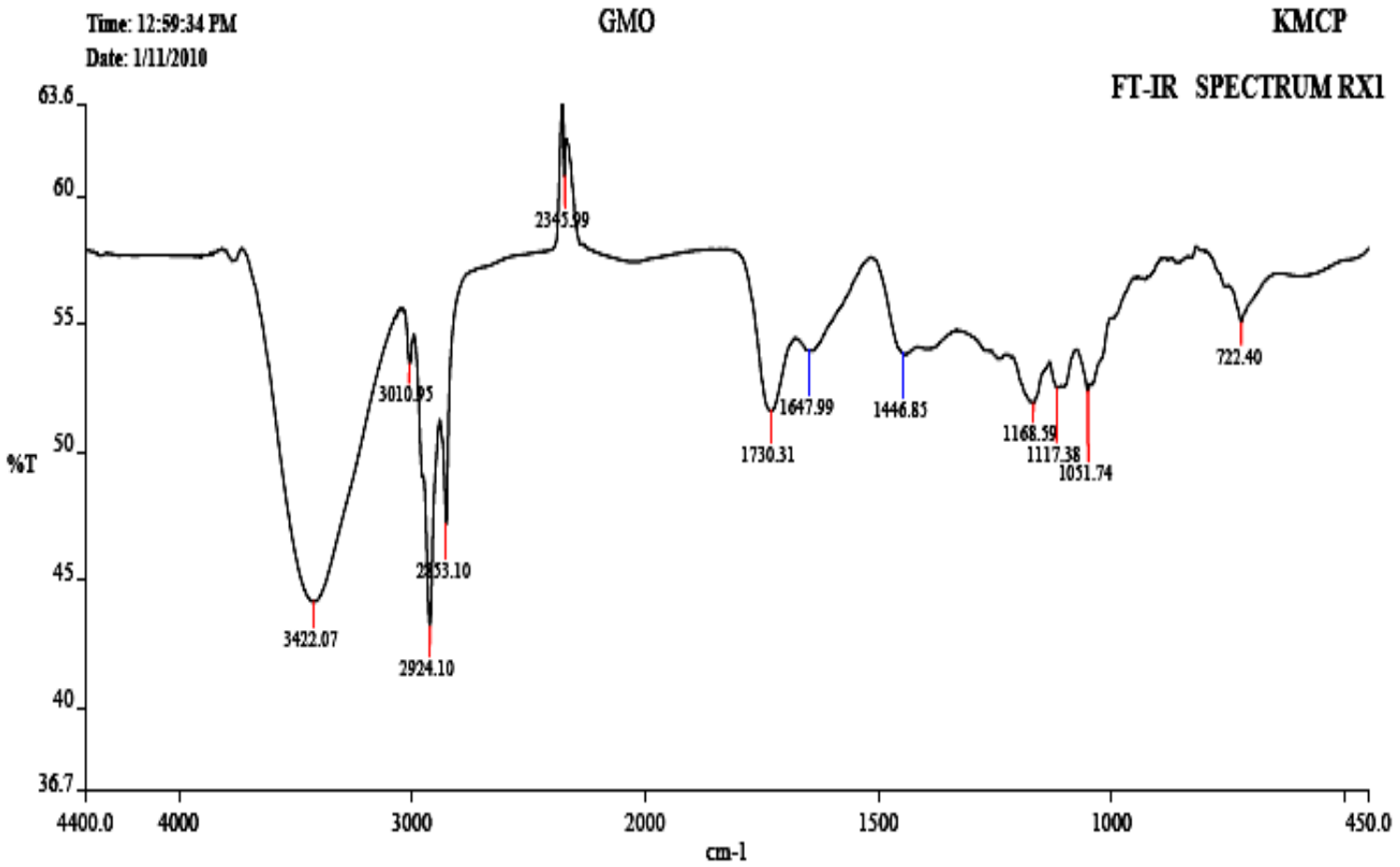
cm-1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%T
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2924.10
2853.10
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1647.99
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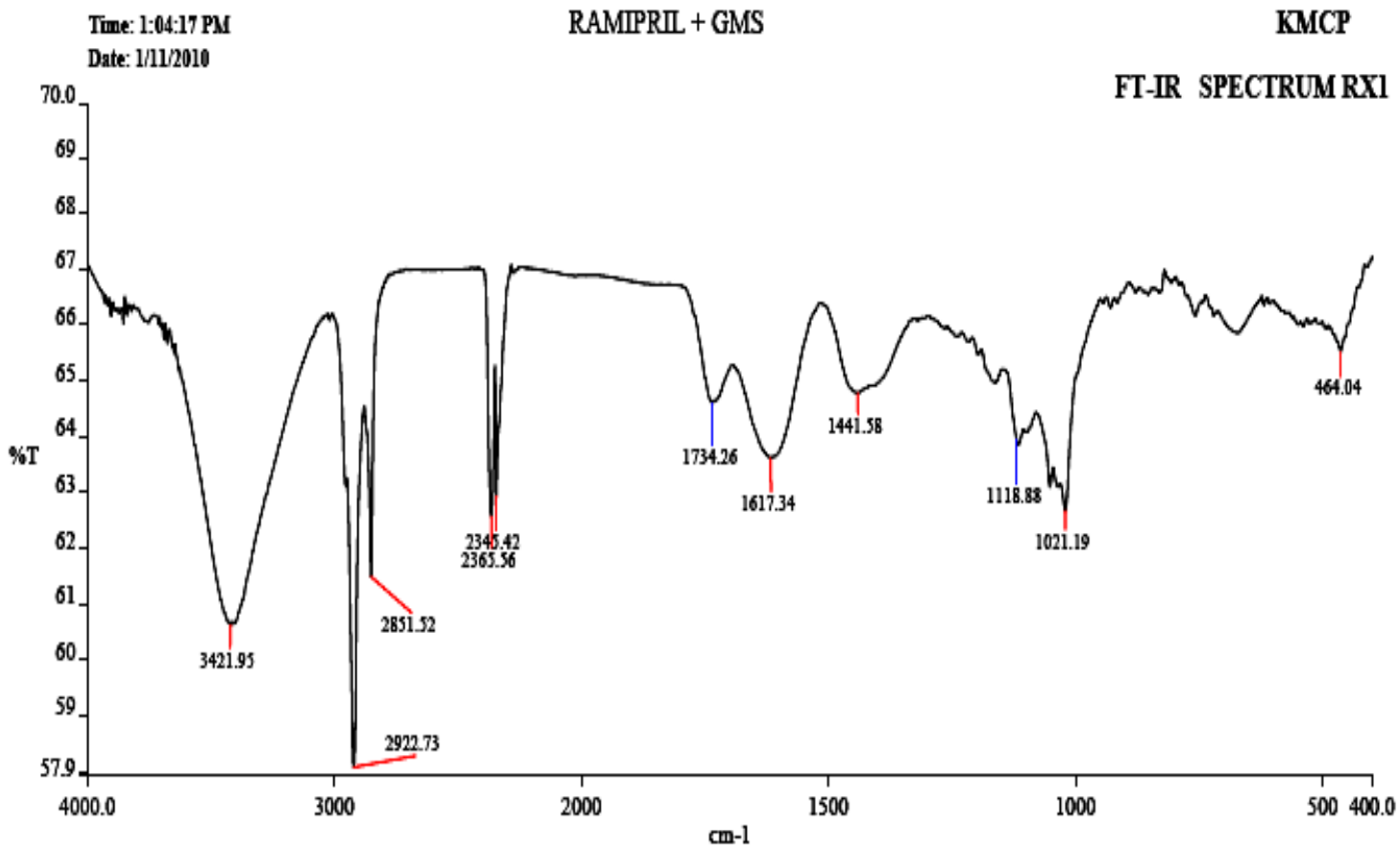
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68
67
66
65
64
63
62
61
60
59
57.9
3421.95
2922.73
2851.52
2345.42
2365.56
1734.26
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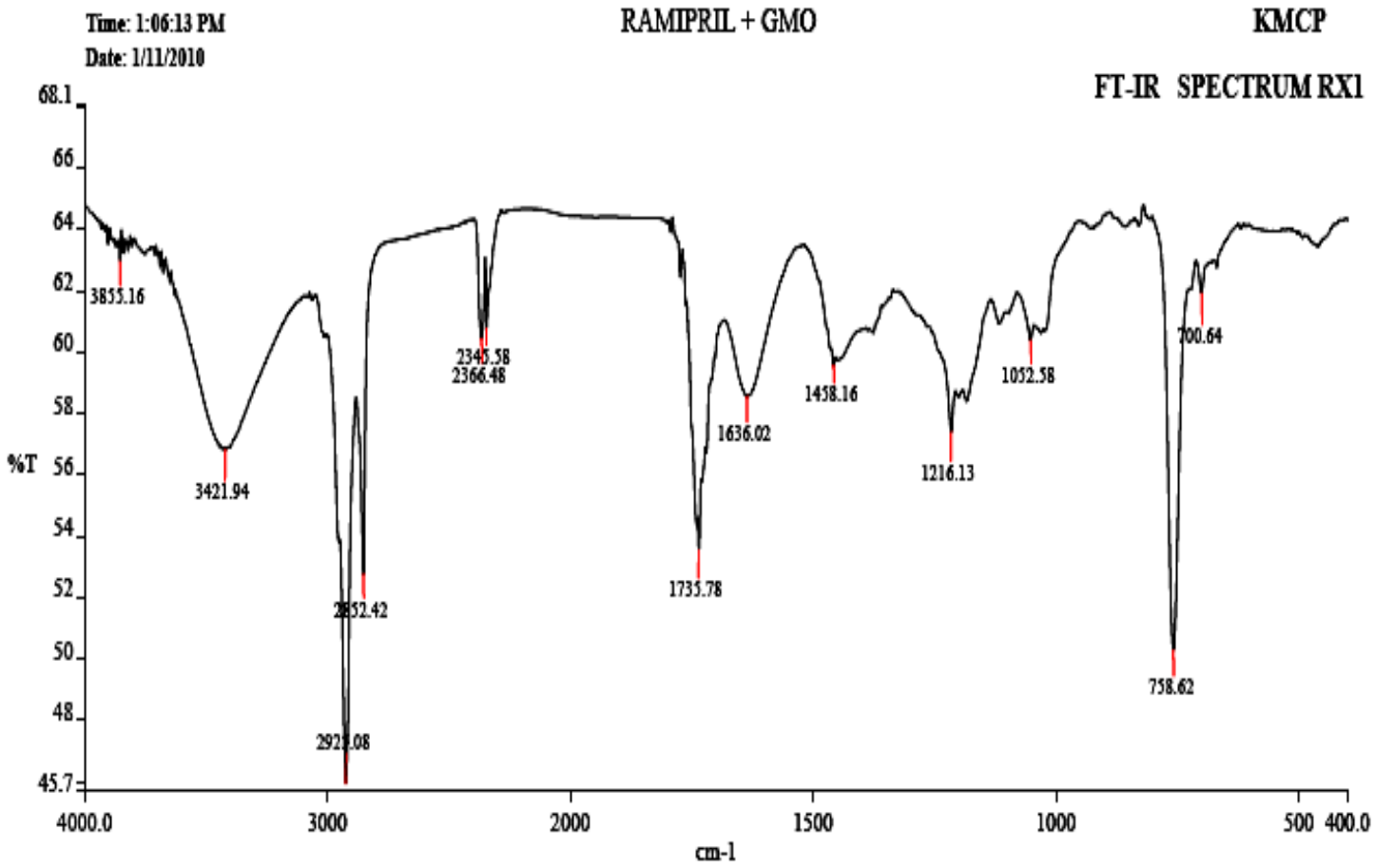
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48
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1735.78
1636.02
1458.16
1216.13
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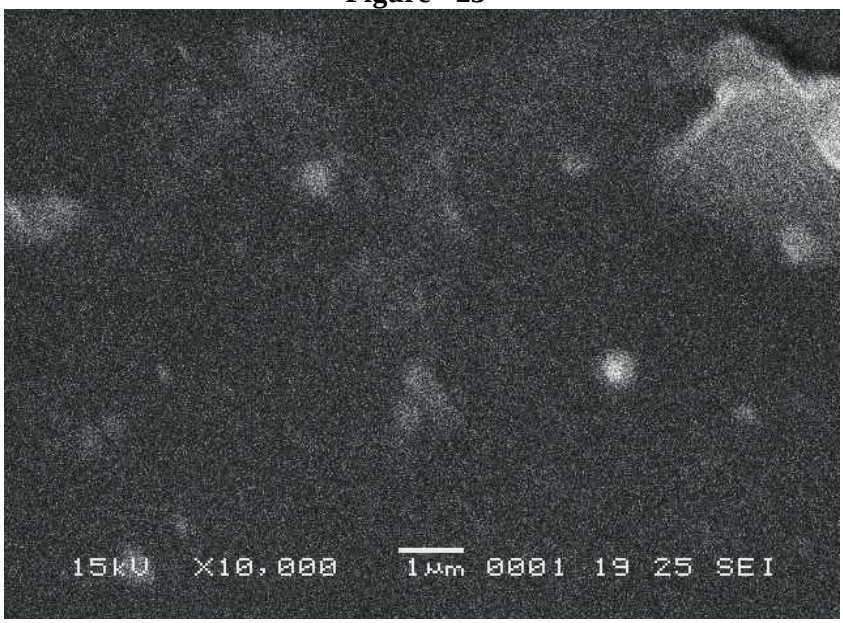
cm-1

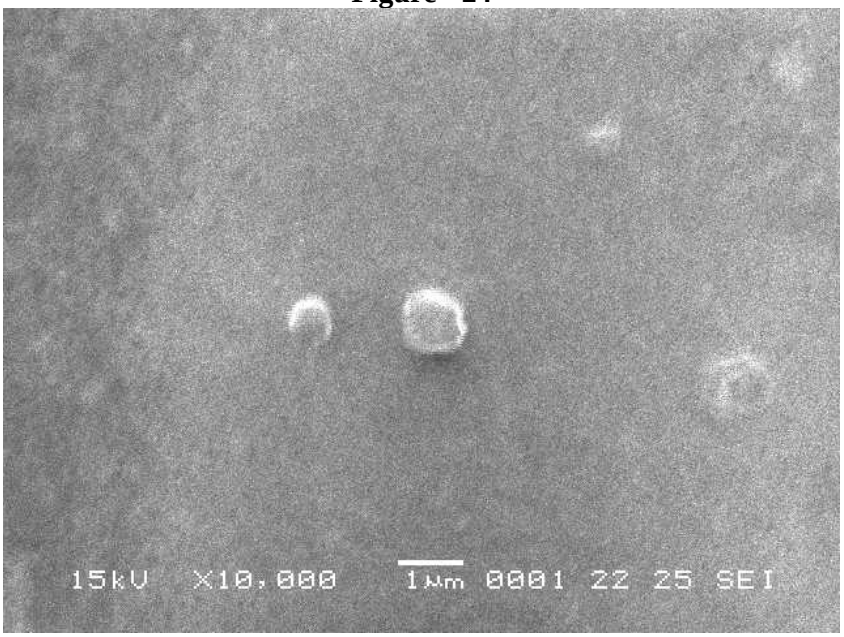
SEM PHOTOGRAPHS

Figure - 23



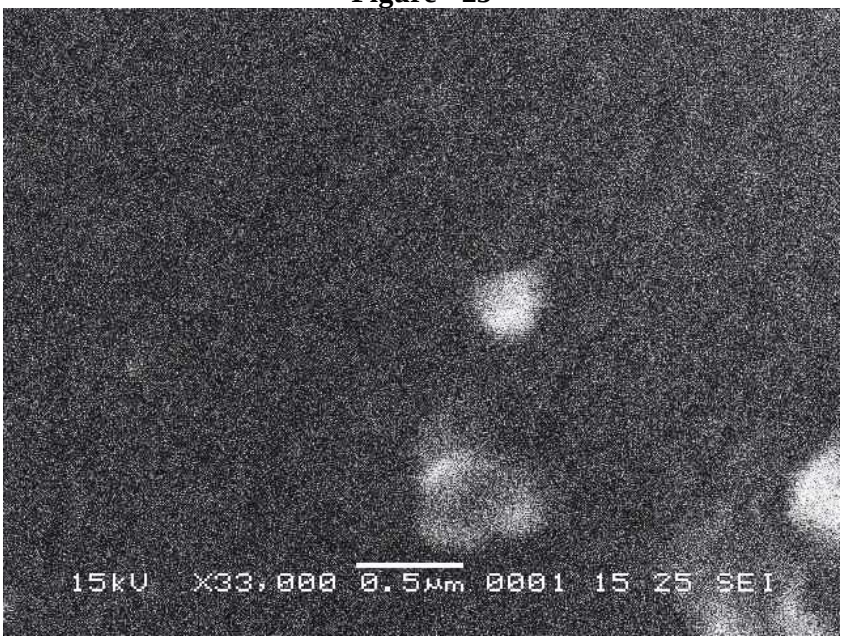
F3 - GMS 6% + Tween 80 2.0%

Figure - 24



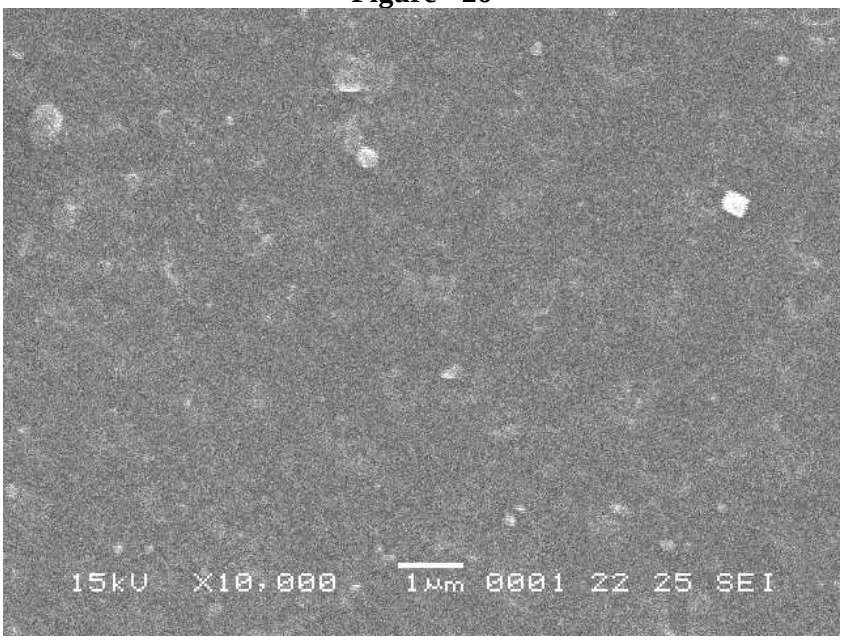
F6 - GMS 6% + Poloxamar 188 2.0%

Figure - 25

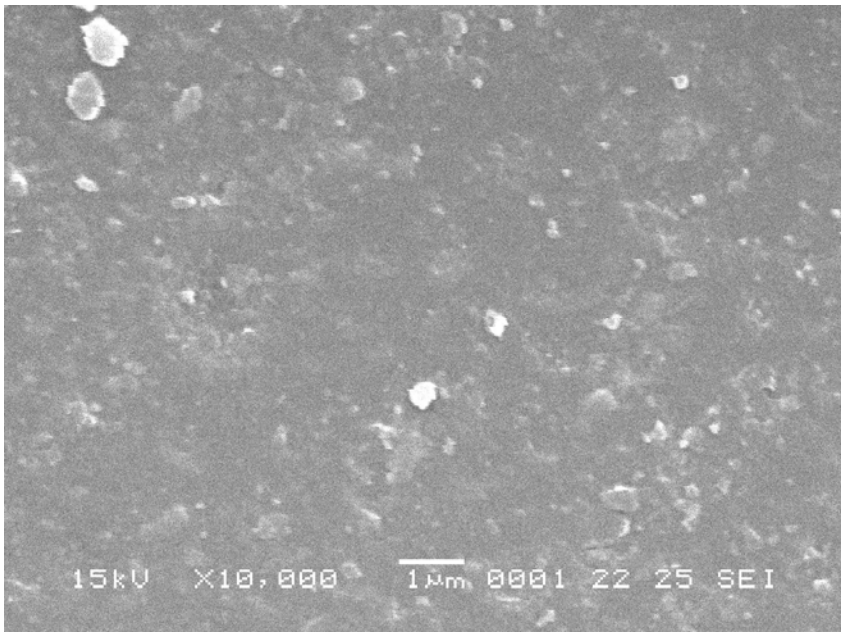


F9 - GMS 6% + Span 20 2.0%

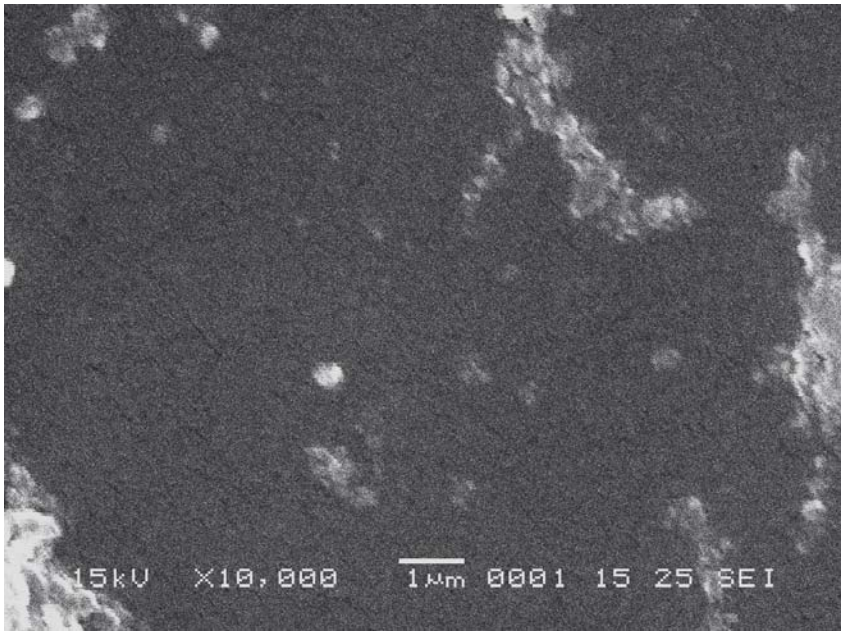
Figure - 26



F12 - GMO 6% + Tween 80 2.0%



F15 - GMO 6% + Poloxamer 188 2.0%



F18 - GMO 6% + Span 20 2.0%

Table -2

CALIBRATION CURVE OF RAMIPRIL IN DISTILLED WATER

S.NO.	**CONCENTRATION (µg/ml)**	**ABSORBANCE** ± SD
1	5	0.111 ± 0.0004
2	10	0.235 ± 0.0008
3	15	0.323 ± 0.0004
4	20	0.45 ± 0.0008
5	25	0.550 ± 0.0004
6	30	0.676 ± 0.0008
7	35	0.765 ± 0.0004
8	40	0.873 ± 0.0004
9	45	0.975 ± 0.0012
10	50	1.075 ± 0.0008

n = 3* γ - 0.999291850

Table -3

CALIBRATION CURVE OF RAMIPRIL IN PHOSPHATE BUFFER SALINE P^H 7.4 BUFFER

S.No.	CONCENTRATION (µg/ml)	ABSORBANCE ± SD
1	5	0.199 ± 0.0009
2	10	0.380 ± 0.0004
3	15	0.583 ± 0.0009
4	20	0.775 ± 0.0031
5	25	0.974 ± 0.0009
6	30	1.172 ± 0.0010
7	35	1.384 ± 0.0009
8	40	1.571 ± 0.0012
9	45	1.762 ± 0.0012
10	50	1.965 ± 0.0012

n = 3* γ - 0.999938578

Table -1

FORMULATION OF RAMIPRIL LOADED SOLID LIPID NANOPARTICLES

S.NO	FORMULATION	COMPOSITION			
		LIPID	% W/V	SURFACTANT	% W/V
1	F1	GMS	6.0	Tween 80	1.0
2	F2	GMS	6.0	Tween 80	1.5
3	F3	GMS	6.0	Tween 80	2.0
4	F4	GMS	6.0	Poloxamer 188	1.0
5	F5	GMS	6.0	Poloxamer 188	1.5
6	F6	GMS	6.0	Poloxamer 188	2.0
7	F7	GMS	6.0	Span 20	1.0
8	F8	GMS	6.0	Span 20	1.5
9	F9	GMS	6.0	Span 20	2.0
10	F10	GMO	6.0	Tween 80	1.0
11	F11	GMO	6.0	Tween 80	1.5
12	F12	GMO	6.0	Tween 80	2.0
13	F13	GMO	6.0	Poloxamer 188	1.0
14	F14	GMO	6.0	Poloxamer 188	1.5
15	F15	GMO	6.0	Poloxamer 188	2.0
16	F16	GMO	6.0	Span 20	1.0
17	F17	GMO	6.0	Span 20	1.5
18	F18	GMO	6.0	Span 20	2.0

GMS – Glyceryl monostearate, GMO – Glyceryl monooleate.
Drug concentration used in each formulation kept as constant 5mg/5ml.

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